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Environment Canada
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Environnement Canada
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Announcement

An author–subject index and list of all publications issued during 1975 will be supplied in early 1976, and distributed free to all *Journal* recipients, including subscribers. Additional copies will be available through the Office of the Editor.

Avis

Un index par sujet et auteur et une liste de toutes les publications parues en 1975 paraîtront au début de 1976 et seront distribués gratuitement à tous ceux qui reçoivent le *Journal*, ainsi qu'aux abonnés. On pourra se procurer des exemplaires supplémentaires au Bureau du directeur de la publication.

J. C. STEVENSON
Editor and/Rédacteur et
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Minimal Dissolved Oxygen Requirements of Aquatic Life with Emphasis on Canadian Species: a Review

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Preface

Originally, some of the material in this report was prepared for the National Research Council of Canada Environmental Secretariat under the title "Waterborne Dissolved Oxygen Requirements and Criteria With Particular Emphasis on the Canadian Environment." That document was published in 1975 as part of N.R.C.'s special publication series under the auspices of the Associate Committee on Scientific Criteria for Environmental Quality. The emphasis in the N.R.C. document was on development of oxygen criteria which were defined as cause and effect interrelations based on a scientific evaluation of environmental harm caused by low oxygen in water. The content of the N.R.C. report was, of necessity, limited to matters considered the mandate of the N.R.C. environmental secretariat.

This report, although similar in many ways to the N.R.C. report, is intended to serve more as a review document to persons interested in oxygen requirements of Canadian aquatic species. Where criteria are discussed, suggestions are made as to how criteria might be implemented. It must be stressed that the suggestions herein are the author's opinions and are not the opinions of N.R.C. whose mandate does not include recommendations on criteria implementation. Readers should be very careful to distinguish between the content and positions taken when making reference to these two reports.

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Abstract

DAVIS, J. C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32: 2295–2332.

This article reviews the sensitivity, responses, response thresholds, and minimum oxygen requirements of marine and freshwater organisms with strong emphasis on Canadian species. The analysis attempts to define low dissolved oxygen thresholds which produce some physiological, behavioral, or other response in different species.

Oxygen availability is discussed with reference to seasonal, geographical, or spatial variation in dissolved oxygen. Factors affecting availability of dissolved oxygen include atmospheric exchange, mixing of water masses, upwelling, respiration, photosynthesis, ice cover, and physical factors such as temperature and salinity. Dissolved oxygen terminology is summarized and tables are included for both fresh and saltwater O_2 solubility at different temperatures.

Incipient O_2 response thresholds are used in a statistical analysis to develop oxygen criteria for safeguarding various groups of freshwater and marine fish. These include mixed freshwater fish populations including or excluding salmonids, freshwater salmonid populations, salmonid larvae or mature salmonid eggs, marine anadromous and nonanadromous species. Criteria are based on threshold oxygen levels which influence fish behavior, blood O_2 saturation, metabolic rate, swimming ability, viability and normal development of eggs and larvae, growth, circulatory dynamics, ventilation, gaseous exchange, and sensitivity to toxic stresses. The criteria provide three levels of protection for each fish group and are expressed as percentage oxygen saturation for a range of seasonal temperature maxima.

Oxygen tolerances and responses of aquatic invertebrates to low oxygen are reviewed for freshwater and marine species according to habitat. No invertebrate criteria are proposed owing to the capacity for many invertebrate species to adopt anaerobic metabolism during low O_2 stress. It is suggested that the criteria proposed for fish species will provide a reasonable safeguard to most invertebrate species. It appears likely, however, that a change in oxygen regime to one of increased O_2 scarcity will probably influence invertebrate community structure.

It is suggested that criteria for protection of aquatic life be implemented by groups of experienced individuals. The group should consider the natural oxygen regime for a specific water body and its natural variability, the aquatic life therein and its value, importance, relative O_2 sensitivity, and the possibility of interactions with toxicants and other factors that may compound the stress produced by low O_2 on aquatic life. Each water body and its aquatic life should be considered as a unique situation and criteria application should not encompass diverse areas, habitats, or biological associations as if they were identical.

Résumé

DAVIS, J. C. 1975. Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review. J. Fish. Res. Board Can. 32: 2295–2332.

Dans cet article, l'auteur passe en revue la sensibilité, les réponses, les seuils de réponse et les exigences minimales en oxygène d'organismes marins et dulçaquicoles, avec accent particulier sur les espèces canadiennes. Il y définit les seuils minimums d'oxygène dissous qui déclenchent des réponses physiologiques, de comportement ou autres chez différentes espèces.

Il analyse la disponibilité d'oxygène en relation avec la variation géographique ou spatiale de l'oxygène. Les facteurs affectant la disponibilité de l'oxygène dissous sont, entre autres, les échanges avec l'atmosphère, le mélange des masses d'eau, la remontée d'eaux profondes, la respiration, la photosynthèse, la couverture de glace et des facteurs physiques tels que la température et la salinité. Il donne un résumé de la terminologie de l'oxygène dissous et des tables de solubilité de l'oxygène, tant en eau douce qu'en eau salée, à différentes températures.

Il utilise les seuils de début de réponse à l'oxygène dans une analyse visant à mettre au point des critères de sauvegarde des divers groupes de poissons de mer et d'eau douce. Ces groupes comprennent des populations mixtes de poissons d'eau douce, incluant ou excluant les salmonidés, des populations de salmonidés d'eau douce, des larves ou des œufs mûrs de salmonidés, et des espèces marines anadromes et non anadromes. Ces critères sont fondés sur les niveaux de seuil d'oxygène affectant le comportement des poissons, la saturation du sang en O_2, le taux métabolique, l'aptitude à la nage, la survivance et le développement normal des œufs et des larves, la croissance, la dynamique de la circulation, les échanges gazeux et la sensibilité aux stress toxiques. Les critères fournissent trois paliers de protection pour chaque groupe de poissons et sont exprimés en pourcentage de saturation d'oxygène sur une gamme de maximums saisonniers de température.

L'auteur passe en revue la tolérance à l'oxygène et les réponses d'invertébrés aquatiques à de bas niveaux d'oxygène, pour des espèces dulçaquicoles et marines, selon l'habitat. Il ne propose aucun critère pour les invertébrés à cause de l'aptitude de plusieurs espèces à adopter un métabolisme anérobie au moment d'un stress causé par un bas niveau d'O_2. Les critères suggérés pour les espèces de poisson fourniraient une sauvegarde raisonnable pour la plupart des espèces

d'invertébrés. Il est probable, cependant, qu'un changement de régime d'O_2 en direction d'une plus grande rareté d'O_2, affecte la structure des communautés d'invertébrés.

L'auteur suggère que les critères de protection de la vie aquatique soient appliqués par des groupes d'individus expérimentés. Ces groupes devraient étudier le régime naturel d'O_2 d'un plan d'eau particulier et sa variabilité naturelle, la sensibilité relative à l'O_2 et la possibilité d'interactions avec les toxiques et autres facteurs qui peuvent s'ajouter au stress causé par un bas niveau d'O_2 sur la vie aquatique. Chaque plan d'eau et la vie aquatique qu'il contient devraient être considérés comme situation unique, et l'application des critères ne devrait pas englober des régions, des associations biologiques et des habitats différents comme s'ils étaient identiques.

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Introduction, Rationale, and Objectives

THE purpose of this paper is to review available information on the oxygen requirements of aquatic organisms as a basis for formulating water oxygen quality criteria that provide a reasonable assessment of hazard for Canadian aquatic resources. A large body of literature exists on the general subject and some excellent reviews have appeared (Doudoroff and Shumway 1970; Warren and Shumway 1964; Warren et al. 1973). This paper attempts to arrive at a consensus of opinion on low oxygen sensitivity of fish and invertebrates. Results of this review are used to develop water quality criteria based on the best available cause and effect information relating to aquatic life.

In preparing this paper it was not intended to reiterate much of the information summarized by other reviewers. Instead, this report attempts to concentrate mainly on aquatic organisms found in Canadian waters, relying on the reader to consult other sources relevant to geographic areas outside Canada. Some considerable attention has been given to defining low oxygen effects on aquatic organisms, identifying oxygen response thresholds, and examining physiological responses to low oxygen under different conditions.

In reviewing the literature it was evident that numerous and often contradictory results have been reported for critical oxygen levels for the same aquatic organisms. This lack of clarity has made it extremely difficult for reviewers to decide the levels of oxygen that are critical as a basis for criteria recommendations. Part of the problem appears to be one of definition. Much of the literature deals with determining lethal levels of dissolved oxygen (see table 1 in Doudoroff and Shumway 1970). In contrast, some workers have defined critical oxygen levels as that point when fish lost equilibrium (e.g. Townsend et al. 1938; Townsend and Earnest 1940; Townsend and Cheyne 1944) or exhibited "respiratory distress" (Wilding 1939). It should be recognized that each of these responses reflects some degree to which the animal responds to low oxygen. Thus there is an entire range of responses to hypoxia (lowered oxygen) which may be exhibited by the test species, and the response varies with the severity of hypoxia. Attempts to arrive at critical oxygen levels, based on data gathered under differing experimental conditions and with varying approaches and experimental criteria, are bound to be frustrating.

The present approach has been to scan the literature and extract information on dissolved oxygen levels where sublethal responses to hypoxia first became apparent (i.e. the incipient sublethal threshold). By extracting this sort of information one becomes aware of the oxygen level where some physiological, behavioral, or other stress-induced response occurs. It is then that the animal indicates at what point energy must be expended or adjustments made to counteract hypoxia. When such a stress is a chronic occurrence, it is an important determinant factor in the long-term survival of an organism.

Some readers may take exception to the above approach, citing the ability of animals to adjust or regulate to altered conditions. A later section will deal with acclimation phenomena, and other sections will explain the significance of observed sublethal responses. It is my belief that we should look for "no-effect" levels in setting water quality criteria that assure the long-term survival of aquatic organisms. Any departure from this level assumes a degree of risk which increases with the severity of hypoxia present. The degree of risk accepted becomes a matter of judgement based on the economic value of an individual aquatic population or an entire aquatic community, assessed against such factors as industrial needs or the aesthetics of unpolluted waters — none of which can be satisfied by scientific criteria.

Physical Considerations

At 19 C fresh water contains 7.93 ml/liter oxygen, roughly 0.8% of its volume, when exposed to air at sea level. Air at the same temperature and pressure, in contrast, holds approximately 209 ml/liter oxygen, roughly 26 times as much oxygen as water. Aquatic organisms have evolved within the physical constraints of their relatively oxygen-scarce environment and many

possess high specialized gas exchange systems which permit maximal utilization of available oxygen supplies.

Aerobic organisms utilize the process of diffusion to move oxygen into their systems by means of an internal–external O_2 pressure gradient. The exchange process is often facilitated by a delivery system (e.g. branchial and opercular pumps in fish) and a transport system (e.g. internal circulatory system) to distribute oxygen within the body. In all aerobic organisms, movement of oxygen to the tissues for metabolism is fundamentally via tension gradients, both within and without the body. Thus, it is the oxygen tension gradient between tissues and the external medium that is critical to the gas exchange process.

Air at sea level contains about 20.9% oxygen. Under standard conditions dry air exerts a pressure equivalent to 760 mm Hg. The oxygen tension can be calculated:

$$PO_2 = (\text{Atmos. Press} - H_2O \text{ vap. press.}) \times \%O_2 \quad (1)$$

where: Atmos. Press. = Atmospheric pressure in millimeters Hg

H_2O vap. press. = water vapor pressure in millimeters Hg at test temperature

$\%O_2$ = percentage oxygen in atmosphere

PO_2 = oxygen partial pressure, millimeters Hg

For the above example at 10 C (assuming Atmos. Press. = 760 mm Hg):

$$PO_2 = (760 - 9.2) \times 20.946\% = 157.3 \text{ mm Hg} \quad (2)$$

Water equilibrated with atmospheric air at sea level usually has an oxygen partial pressure of about 154–158 mm Hg. Thus organisms exposed to fully saturated water under those conditions exist with an external oxygen pressure of 154–158 mm Hg. In fish the internal oxygen tension (ca. 50–110) is lower than the external tension. Oxygen therefore diffuses across the gills into the blood down an O_2 gradient of 40–100 mm Hg (Randall 1970). A drop of external PO_2 reduces this gradient or depresses arterial PO_2 levels. If the oxygen depression is severe, insufficient O_2 tension will occur at the tissue level.

For practical purposes the PO_2 of water can be calculated from the above cited equation (1), when the barometric pressure is known. Water vapor pressure can be obtained from tables (e.g. Table 1) for purposes of calculation.

It is more conventional for water quality or oceanographic purposes to express water oxygen levels in terms of their concentration rather than partial pressure. Thus one speaks of oxygen in terms of milliliters O_2/liter or mg O_2/liter (parts per million). All the terms can be interrelated such that a sample of air-equilibrated fresh water at 10 C at sea level has 11.33 mg O_2/liter (ppm), 7.93 ml O_2/liter (1 mg O_2 = 0.7 ml O_2) and from (2) has a partial pressure of 157.3 mm Hg. If that sample were only 50% saturated, it would have 11.33/2 = 5.67 mg O_2/liter, 7.93/2 = 3.82 ml O_2/liter, and a PO_2 of 157.3/2 = 78.65 mm Hg.

As temperature and salinity increase, oxygen content decreases. Tabulated values (Tables 2 and 3) and well-known formulae (Gameson and Robertson 1955; Truesdale and Gameson 1957) enable easy calculation of oxygen content of water at different temperatures and salinities. As temperature increases, oxygen content drops

TABLE 1. Saturated water vapor pressure and oxygen partial pressures in relation to water temperature in water exposed to fully oxygen-saturated air at 760 mm Hg air pressure.

Temp (C)	Saturated water vapor pressure (*in mm Hg*)	Oxygen partial pressure for 100% air saturation at 760 mm Hg pressure in water (*in mm Hg*)
−5	3.0	158.6
−4	3.4	158.5
−3	3.7	158.4
−2	4.0	158.4
−1	4.3	158.3
0	4.6	158.3
+1	4.9	158.2
+2	5.3	158.1
+3	5.7	158.0
+4	6.1	157.9
+5	6.5	157.9
+6	7.0	157.8
+7	7.5	157.6
+8	8.0	157.5
+9	8.6	157.4
+10	9.2	157.3
+11	9.8	157.2
+12	10.5	157.0
+13	11.2	156.9
+14	12.0	156.7
+15	12.8	156.5
+16	13.6	156.4
+17	14.5	156.2
+18	15.5	156.0
+19	16.5	155.8
+20	17.5	155.6
+21	18.6	155.3
+22	19.8	155.1
+23	21.0	154.8
+24	22.4	154.5
+25	23.7	154.3
+26	25.2	153.9
+27	26.7	153.6
+28	28.3	153.3
+29	30.0	152.9
+30	31.8	152.6

TABLE 2. Solubility of oxygen in fresh water at different temperatures when water is exposed to an atmosphere containing 20.9% oxygen at a pressure of 760 mm Hg (including water vapor pressure) (derived from Whipple 1914).

Temp (C)	Parts per million	Cm^3 per liter (*at 0 C and 760 mm*)	Temp (C)	Parts per million	Cm^3 per liter (*at 0 C and 760 mm*)
0	14.62	10.23	16	9.95	6.96
1	14.23	9.96	17	9.74	6.82
2	13.84	9.68	18	9.54	6.68
3	13.48	9.43	19	9.35	6.54
4	13.13	9.19	20	9.17	6.42
5	12.80	8.96	21	8.99	6.29
6	12.48	8.73	22	8.83	6.18
7	12.17	8.52	23	8.68	6.07
8	11.87	8.31	24	8.53	5.97
9	11.59	8.11	25	8.38	5.86
10	11.33	7.93	26	8.22	5.75
11	11.08	7.75	27	8.07	5.65
12	10.83	7.58	28	7.92	5.54
13	10.60	7.42	29	7.77	5.44
14	10.37	7.26	30	7.63	5.34
15	10.15	7.10			

owing to reduced solubility, while oxygen partial pressure drops only slightly owing to increased molecular activity. Thus a fish breathing warm water must pump more water over the gills than in cold water to deliver a given volume of oxygen/unit time, because of the reduced oxygen content of inspired water. This is necessary even though the O_2 tension gradient between blood and water is little changed at high temperatures. Severe respiratory problems can result from a combination of high temperature (low O_2 content) and reduced oxygen tension (water not fully saturated) as both availability and the gradient for oxygen diffusion are reduced. Associated with this phenomenon is the fact that higher temperatures usually increase the metabolic demand for oxygen. It is likely that the stimulatory effect of increased temperature on metabolism is the most important of all these factors owing to the magnitude of its effect. It is largely for these reasons that fish kills related to low oxygen levels often occur in warmwater periods. Obviously, meaningful oxygen criteria should consider the critical oxygen content and oxygen tension as well as temperature effects on metabolism in order to provide a full measure of protection.

Natural O_2 Regimes in the Aquatic Environment

Bodies of fresh water often show large variations in dissolved oxygen, both seasonally and geographically. Welch (1952) attributed to photosynthesis values of oxygen supersaturation as high as 364.5% in the upper portion of a Wisconsin lake. Tarzwell and Gaufin (1953) described a location in a southern Ohio creek polluted with sewage where dissolved oxygen was 19.4 mg O_2/liter one afternoon and 0.7 mg O_2/liter the next morning. Oxygen concentrations in ponds and lakes exceeding 30 mg O_2/liter have been reported (Wiebe 1933; Woodbury 1942). Servizi and Burkhalter (1970) reported oxygen levels close to saturation on the surface of the Thompson and Fraser rivers in 1963 and 1966, prior to operation of pulp mills on the upper reaches of the rivers. Seasonally low levels of oxygen in freshwater bodies can lead to "winter kills" resulting from decay of organic materials under ice cover without replenishment of oxygen from the atmosphere. Summer kills can result from high water temperatures, reduced water oxygen solubility, and large oxygen demands from planktonic and bacterial respiration.

In the sea, oxygen levels in the photic zone may rise to 130% saturation or higher, owing to photosynthetic processes (Fairbridge 1966). As well as the contribution from photosynthesis, oxygen enrichment occurs in water by atmospheric exchange. The rate of enrichment is dependent on the rate of "turnover" of the system. In deep marine waters, oxygen levels are generally lower than at the surface owing to reduced atmospheric exchange and to conditions where respiration exceeds photosynthesis. Fairbridge (1966) reported that Atlantic bottom water generally does not fall below 60% saturation, while oxygen levels in deep Pacific water may be somewhat lower. In

TABLE 3. Solubility of oxygen in sea water of salinity 35‰, in milligrams per liter, from a normal atmosphere saturated with water vapor at a total pressure of 760 mm of mercury from −2 to +40 C. ΔC_1 is the change in solubility for a change of 1‰ in salinity (from Truesdale and Gameson 1957). (This table can be used to calculate oxygen content of water at various temperatures and salinities.)

Temp (C)	.9	.8	.7	.6	.5	.4	.3	.2	.1	.0	ΔC_1
−2										11.86	
−1	11.82	11.79	11.76	11.72	11.69	11.66	11.63	11.59	11.56	11.53	−0.088
−0	11.50	11.47	11.44	11.41	11.37	11.34	11.31	11.28	11.25	11.22	−0.085

Temp (C)	.0	.1	.2	.3	.4	.6	.6	.7	.8	.9	ΔC_1
+0	11.22	11.19	11.16	11.13	11.10	11.07	11.04	11.01	10.98	10.95	−0.083
1	10.92	10.89	10.86	10.83	10.80	10.78	10.75	10.72	10.69	10.66	−0.080
2	10.64	10.61	10.58	10.55	10.52	10.50	10.47	10.44	10.41	10.39	−0.078
3	10.36	10.33	10.31	10.28	10.26	10.23	10.20	10.18	10.15	10.12	−0.076
4	10.10	10.07	10.05	10.02	10.00	9.97	9.95	9.92	9.90	9.87	−0.073
5	9.85	9.82	9.80	9.78	9.75	9.73	9.70	9.68	9.66	9.63	−0.071
6	9.61	9.58	9.56	9.54	9.52	9.49	9.47	9.45	9.42	9.40	−0.069
7	9.38	9.36	9.33	9.31	9.29	9.27	9.25	9.22	9.20	9.18	−0.067
8	9.16	9.14	9.12	9.10	9.07	9.05	9.03	9.01	8.99	8.97	−0.065
9	8.95	8.93	8.91	8.89	8.87	8.85	8.83	8.81	8.79	8.77	−0.063
10	8.75	8.73	8.71	8.69	8.67	8.65	8.63	8.61	8.59	8.58	−0.061
11	8.56	8.54	8.52	8.50	8.48	8.46	8.45	8.43	8.41	8.39	−0.059
12	8.37	8.36	8.34	8.32	8.30	8.29	8.27	8.25	8.23	8.22	−0.058
13	8.20	8.18	8.17	8.15	8.13	8.12	8.10	8.08	8.06	8.05	−0.056
14	8.03	8.02	8.00	7.98	7.97	7.95	7.94	7.92	7.90	7.89	−0.055
15	7.87	7.86	7.84	7.83	8.81	7.80	7.78	7.76	7.75	7.73	−0.053
16	7.72	7.70	7.69	7.67	7.66	7.65	7.63	7.62	7.60	7.59	−0.052
17	7.57	7.56	7.54	7.53	7.52	7.50	7.49	7.48	7.46	7.45	−0.051
18	7.43	7.42	7.41	7.39	7.38	7.37	7.35	7.34	7.33	7.31	−0.049
19	7.30	7.29	7.27	7.26	7.25	7.23	7.22	7.21	7.20	7.18	−0.048
20	7.17	7.16	7.15	7.13	7.12	7.11	7.09	7.08	7.07	7.06	−0.047
21	7.05	7.03	7.02	7.01	7.00	6.99	6.97	6.96	6.95	6.94	−0.046
22	6.93	6.92	6.90	6.89	6.88	6.87	6.86	6.85	6.84	6.82	−0.045
23	6.81	6.80	6.70	6.78	6.77	6.76	6.75	6.73	6.72	6.71	−0.044
24	6.70	6.69	6.68	6.67	6.66	6.65	6.64	6.63	6.62	6.61	−0.044
25	6.60	6.59	6.57	6.56	6.55	6.54	6.53	6.52	6.51	6.50	−0.043
26	6.49	6.48	6.47	6.46	6.45	6.44	6.43	6.42	6.41	6.40	−0.042
27	6.39	6.38	6.37	6.36	6.35	6.34	6.33	6.32	6.31	6.30	−0.042
28	6.29	6.28	6.27	6.26	6.25	6.24	6.23	6.22	6.21	6.20	−0.041
29	6.19	6.18	6.17	6.16	6.15	6.14	6.13	6.12	6.12	6.11	−0.041
30	6.10	6.09	6.08	6.07	6.06	6.05	6.04	6.03	6.02	6.01	−0.041
31	6.00	5.99	5.98	5.97	5.97	5.96	5.95	5.94	5.93	5.92	−0.040
32	5.91	5.90	5.89	5.88	5.87	5.86	5.85	5.84	5.83	5.82	−0.040
33	5.82	5.81	5.80	5.79	5.78	5.77	5.76	5.75	5.74	5.73	−0.040
34	5.72	5.71	5.70	5.69	5.68	5.68	5.67	5.66	5.65	5.64	−0.040
35	5.63	5.62	5.61	5.60	5.59	5.58	5.57	5.56	5.55	5.54	−0.040
36	5.53	5.52	5.51	5.51	5.50	5.49	5.48	5.47	5.46	5.45	−0.040
37	5.44	5.43	5.42	5.41	5.40	5.39	5.38	5.37	5.36	5.35	−0.041
38	5.34	5.33	5.32	5.31	5.30	5.29	5.28	5.27	5.26	5.25	−0.041
39	5.24	5.23	5.22	5.21	5.20	5.19	5.18	5.17	5.16	5.15	−0.041
40	5.14										

coastal Atlantic inlets such as Bedford Basin (Krauel 1969) and Petpeswick Inlet (Platt and Irwin 1972), surface water is close to saturation on a year-round basis while bottom water can have seasonally low oxygen levels of 40 and 0% saturation in the two areas, respectively.

Pickard (1961, 1963) provided a comprehensive study of the oceanographic characteristics of a number of mainland British Columbia and Vancouver Island inlets. These areas are important to commercial and recreational fisheries. They are also attractive as sites for location of oxygen-depleting industries such as pulp mills. In general, oxygen levels are high in the surface waters of

these inlets, whereas the deep inlet water is often low in oxygen.

In Vancouver Island inlet studies, Pickard encountered considerable variability in dissolved oxygen. He found vertical stratification related to temperature and salinity variance. Horizontal oxygen stratification was due to pockets of photosynthesis, or respiration and decay or organics. At depths greater than 100 m, oxygen levels were usually less than 4 ml O_2/liter (5.7 mg/liter) and in many instances they were below 1 ml O_2/liter (1.4 mg/liter).

Mainland B.C. inlets receive considerable freshwater runoff of varying volume flow, chiefly from glaciers. On this topic, Pickard (1961) has stated:

"Generally the large-runoff inlets show less variable dissolved oxygen values along an inlet at any depth than do small runoff inlets. Supersaturation of the upper layers is common and there is often an oxygen maximum just below the halocline of the larger-runoff inlets. A few small-runoff inlets have a mid-depth oxygen minimum in which the lowest values are at the inlet head. Dissolved oxygen values of less than 2 ml/l are not common in any mainland inlets and zero values have not been definitely recorded."

Useful dissolved oxygen profiles for B.C. inlets are shown in Pickard (1961, 1963).

Oxygen Requirements of Fish

A summary of literature values of incipient oxygen response thresholds for Canadian aquatic life appears in Table 4A–D. A detailed description of the summarized responses and the rationale for including each of them follows.

Blood Oxygen Dissociation Curves

The process of diffusion of oxygen into the blood depends on a partial pressure difference, describing a gradient between blood and water. In many fish the respiratory pigment hemoglobin has a high affinity for oxygen which enables it to act as an oxygen transport mechanism. The blood oxygen dissociation curve for fish relates the percentage saturation of the blood to the oxygen partial pressure (PO_2) applied. The hemoglobin curve maintains the gradient between water and blood, and blood and tissues, and therefore promotes a high rate of gas transfer.

Figure 1 illustrates that rainbow trout blood remains nearly 100% saturated with oxygen until PO_2 drops below 80 mm Hg. These data also indicate how an increase in either temperature or CO_2 tension shifts the curve to the right. Arterial blood in fish has a low PCO_2 owing to the high water solubility of CO_2 and ease of CO_2 loss via the gills (Randall 1970). In the tissues, higher CO_2 levels shift the blood curve to the right causing release of oxygen.

The oxygen tension where the blood ceases to be fully oxygen saturated is indicative of a limiting oxygen condition for fish. Under these conditions more circulatory and ventilatory work must be done to meet the oxygen requirements of the tissues. Indeed, even at PO_2's above the point where arterial blood ceases to be saturated as PO_2 drops, a critical point is reached owing to the need to maintain a gradient between water and blood to drive oxygen into the blood. Jones et al. (1970), on the basis of data for rainbow trout at 5 C, suggested that a PO_2 of 118 mm Hg was necessary to maintain a proper gradient for oxygen uptake. Randall (1970) calculated that the internal–external gradient for trout should be 20 mm Hg PO_2. C. M. Wood (unpublished data) noted physiological signs of hypoxia 20 mm Hg PO_2 above the point where the blood ceased to be fully saturated. This relationship was related to the shape of the blood curve such that when it shifted with temperature so did the PO_2 threshold for hypoxia responses.

Ventilation, Circulation, and Respiratory Dependence

Ventilatory and circulatory changes have been observed in a number of fish species in response to hypoxia. Rainbow trout (Holeton and Randall 1967a, b) and carp (Garey 1967) show elevations in both rate and amplitude of breathing and decreased heart rate in response to low oxygen. In trout the stroke volume output of the heart also increases, thus maintaining cardiac output as heart rate decreases. The gill transfer factor for oxygen (a measure of the ability of the gills to transfer oxygen per unit gradient) increases, gill water flow goes up, and venous oxygen tension drops. All these factors operate to maintain oxygen uptake in the face of reduced oxygen availability.

In understanding effects of low oxygen on metabolism, it is useful to refer to Fig. 2 which embodies the classical concepts of Fry (1957). Metabolic rate (oxygen uptake rate) is shown plotted against oxygen tension. Standard metabolic rate was defined by Brett (1962) as the minimal metabolic rate accompanying the energy cost of maintenance, measured under laboratory conditions. It is close to, but slightly higher than, the term "basal metabolic rate" used by other workers. The point at which available oxygen level coincides with the oxygen needs for bare maintenance of bodily functions is termed the

TABLE 4. The incipient oxygen response thresholds for various fish groups and habitats gathered from the literature with emphasis on Canadian species. The rationales for choosing the reported response and its significance are reported in the following pages 2301–2311. In most instances, oxygen response thresholds were found in only one form of terminology (e.g. column 6 — mg O_2/liter and the others in the table were calculated. Calculations for freshwater fish made use of the reported temperature and the O_2 solubility data in Tables 1 and 2 while those for nonanadromous fish in sea water used the data in Table 3 and an assumed salinity of 28‰. It is stressed that the response levels reported are incipient oxygen levels where effects first become apparent thus providing a "biological indication" of the onset of hypoxic stress. A) Freshwater species, B) Marine nonanadromous species, C) Anadromous species, D) Eggs and larvae (freshwater and marine species).

Species	Size	Temp (C)	Level of dissolved oxygen				Response to oxygen level	Reference
			PO_2-mm Hg	ml O_2/liter	mg O_2/liter	% Satn.		
A. Freshwater species								
Arctic char, *Salvelinus alpinus*	—	2± .05	25	1.53	2.18	15.8	"signs of asphyxia and loss of equilibrium"	Holeton (1973)
brown bullhead, *Ictalurus nebulosus*	43–127 g	9–10	60	4.87–4.76	6.95–6.80	38.0	onset of oxygen — dependent oxygen uptake	Grigg (1969)
Rainbow trout, *Salmo gairdneri*	682–1136 g	20	78	3.20	4.59	50	below this level blood is not fully saturated with oxygen	Irving et al. (1941)
" "	120–250 g	8.5–15	80–100	3.63–5.14	5.18—7.34	50.7–63.7	circulatory changes occur, including a slowing of heart	Randall and Smith (1967)
carp, *Cyprinus carpio*	77–350 g	10	50	2.51	3.59	31.7	standard oxygen uptake elevated (depressed at lower levels)	Beamish (1964)
	30–600 g	20	80	3.29	4.71	51.3		
" "	235–785 g	13.3–25	80	2.74–3.04	5.39–4.34	50.9–51.6	blood ceased to be fully saturated with O_2 below this level	Itazawa (1970)
Walleye, *Stizostedion vitreum*	2 yr-old	22	35.2–70.4	2.8–1.4	4–2.0	45.3–22.7	loss of negative phototaxis (light avoidance behavior)	Scherer (1971)
			70.4–96.8	3.85–2.8	5.5–4.0	45.3–62.3	increased mobility, "darting" behavior	
Largemouth bass, *Micropterus salmonides*	5–9 cm	—	—	3.15	4.5	—	some indication of avoidance behavior	Whitmore et al. (1960)
				1.1	1.5	—	marked avoidance	
Brook trout (speckled trout) *Salvelinus fontinalis*	682–1136 g	20	77.95	3.21	4.59	50	blood ceases to be fully satd. with O_2 below this level	Irving et al. (1941)
" "	17–65 g	5	100	5.66	8.09	63.2	onset of O_2-dependent metabolism	Graham (1949)
		8	80	4.19	5.99	50.7	reduced cruising speed	
		20	154	6.34	9.06	98.8	onset of O_2 dependent metabolism	
		5–20	116.9–118.7	6.72–4.82	9.6–6.88	75	reduced activity, all temps.	
" "	56–140 g	10,15	80	4.03–3.63	5.75–5.18	50.7–51.0	standard oxygen uptake reduced below this level	Beamish (1964)
Brown trout, *Salmo trutta*	682–1136 g	20	77.95	3.21	4.59	50	blood ceases to be fully satd. with O_2 below this level	Irving et al. (1941)
Rainbow trout, *Salmo gairdneri*	13.3±1.4 cm	17± .5	156.6	6.82	9.74	100	any reduction in oxygen led to more rapid death in cyanide	Downing (1954)
" "	20 mos old	8–10	78.85–79.95	4.16–3.97	5.94–5.67	50	43% reduction in maximum swimming speed	Jones (1971b)
		21–23	77.85–77.6	3.15–3.04	4.50–4.34	50	30% reduction in maximum swimming speed	
" "	235–510 g	2.3–13	100	6.11–4.71	8.73–6.74	63.1–63.6	blood is not fully satd. with oxygen below this level	Itazawa (1970)
" "	—	15	78.45	3.55	5.08	50	altered respiratory quotient, little capacity for anaerobic metabolism below this level	Kutty (1968a)
	—	15	80–100	3.63–4.53	5.18–6.47	51.0–63.7	changes in oxygen transfer factor and effectiveness of O_2 exchange occur	Randall et al. (1967)

TABLE 4. (*Continued*)

Species	Size	Temp (C)	Level of dissolved oxygen				Response to oxygen level	Reference
			PO_2-mm Hg	ml O_2/liter	mg O_2/liter	% Satn.		
" "	400–600 g	13.5	80	3.74	5.35	51.0	breathing amplitude and buccal pressure elevated	Hughes and Saunders (1970)
" "	approx. 300 g	10,15, 20	80	3.30–4.03	4.71–5.75	50.7–51.3	below this level blood is not fully satd. with oxygen	Cameron (1971)
" "	1–11 g	17.5	93.8	4.05	5.78	60	toxicity of zinc, lead, copper, phenols increased markedly below this level	Lloyd (1961)
Largemouth bass, *Micropterus salmoides*	juvenile	25	92.3–110.7	3.5–4.2	5–6	59.7–71.6	final (maximum sustained) swimming speed reduced below this level	Dahlberg et al. (1968)
Bluegill, *Lepomis macrochirus*	5–9 cm	—	—	2.1	3.0	—	some avoidance behavior	Whitmore et al. (1960)
				1.1	1.5	—	strong avoidance	
B. Marine nonanadromous species								
Pile perch, *Rhacochilus vacca*	adults	11	80	3.19	4.56	50.8	blood ceases to be fully O_2 saturated below this level	Webb and Brett (1972)
Dogfish, *Squalus suckleyi*	2.5–6.0 kg	10	115	4.68	6.69	72.92	blood ceases to be fully O_2 saturated below this level	Lenfant and Johansen (1966)
Dogfish, *Scyliorhinus canicula*[a]	150–600 g	12–14	80	3.12–2.95	4.46–4.20	50.8–51.0	Oxygen-dependent metabolism begins below this level	Hughes and Umezawa (1968a)
Dragonet, *Callionymus lyra*[a]	70–140 g	11–12	125	4.98–4.88	7.12–6.97	79.4	Oxygen-dependent metabolism below this level	Hughes and Umezawa (1968b)
			100	3.97–3.90	5.67–5.58	63.5	altered heart rate	
Dragonet, *Callionymus lyra*[a]	80–120	—	80	—	—	—	increased ventilatory muscle activity below this level	Hughes and Ballintijn (1968)
Ratfish, *Hydrolagus colliei*	—	11	150	5.98	8.54	95.2	blood ceases to be fully O_2 saturated below this level	Hanson (1967)
Atlantic cod, *Gadus morhua*	1.14–2.33 kg	5	158.2	7.14	10.20	100	any reduction in ambient O_2 level produces rise in ventilatory water flow	Saunders (1963)
C. Anadromous species								
Steelhead, *Salmo gairdneri* —	*see freshwater species*							
Sockeye salmon, *Oncorhynchus nerka*	50 g	20–24	155.9–154.9	6.42–5.97	9.17–8.53	100	available oxygen level appears to limit active metabolism and maximum swimming speed	Brett (1964)
" "	1579 g	13	100	4.72	6.74	63.6	blood ceases to be fully O_2 saturated below this level	Davis (1973)
" "	1.5–1.7 kg	15	78.45	3.55	5.07	50	elevated blood and buccal pressure and breathing rate increased	Randall and Smith (1967)
Coho salmon, *Oncorhynchus kisutch*	6.3–11 cm	"summer temperatures"	—	3.15	4.5	—	erratic avoidance behavior	Whitmore et al. (1960)
	5.1–14.8 cm	12±1	130.8	6.3	9.0	83.1	below this level acute mortality in kraft pulpmill effluent increased	Hicks and DeWitt (1971)
" "	Juvenile	10–20	157.7–155.9	7.93–6.42	11.33–9.17	100	reduction of O_2 below saturation produced some lowering of maximum sustained swimming speed	Davis et al. (1963)
" "	"	20	155.9	6.42	9.17	100	as above	Dahlberg et al. (1968)
" "	"	"	136–67.9	5.6–2.8	8.0–4.0	87.2–43.6	growth rate proportional to oxygen level with best growth at 8.0 mg/liter, lowest at 4.0 mg/liter	Hermann (1958)

TABLE 4. (*Concluded*)

Species	Size	Temp (C)	Level of dissolved oxygen				Response to oxygen level	Reference
			PO_2-mm Hg	ml O_2/liter	mg O_2/liter	% Satn.		
Chinook salmon, *Oncorhynchus tshawytscha*	6.3–11 cm	"summer temps"	—	3.15	4.5	—	marked avoidance of this level in summer	Whitmore et al. (1960)
		"fall temps"	—	—	4.5	—	little avoidance of this level in fall	
" "	Juveniles	10–20	157.7–155.9	7.93–6.42	11.33–9.17	100	reduction of O_2 below saturation produced some lowering of maximal sustained swimming speed	Davis et al. (1963)
Atlantic salmon, *Salmo salar*	87–135 g	15	69.6	3.15	4.5	44.33	Salmon stop swimming at a speed of 55 cm/s at O_2 levels below this. Faster swimming requires more oxygen	Kutty and Saunders (1973)
American shad, *Alosa sapidissima*	—	—	—	1.75–2.1	2.5–3.0	—	threshold for migration through polluted areas	Chittenden (1973)
				2.8	4.0	—	necessary for spawning areas	
			D. Eggs and larvae					
Rainbow trout, larvae, *Salmo gairdneri*	1+8 day old larvae	10+1	100	5.03	7.18	63.4	elevated heart and breathing rates below this level. Bradycardia (heart rate slows) sets in at lower O_2 levels	Holeton (1971)
Chum salmon eggs, *Oncorhynchus keta*	early stage eggs	10	13.9	.7	1	8.8	retarded development, deformities, mortality, respiratory O_2-dependence	Alderdice et al. (1958)
	pre-hatch eggs	10	>97.4	>4.9	>7.0	>61.8	oxygen level required for normal development and non O_2-dependent metabolism	
" "	5 day eggs	8.0–8.2	22.2	1.17	1.67	14.07	critical oxygen level for supply of oxygen demand and non O_2-dependent metabolism	Wickett (1954)
	(eyed) 85 day eggs	3.6–4.9	>60.3[b]	>3.5[b]	>5.0	>38.1[b]		
Atlantic salmon, *Salmo salar*	early eggs	5.5	9.62	.53	0.76	6.09	As above	Lindroth (1942) (from Wickett 1954))
	near hatching	5	71.7	4.06	5.80	45.31		
	hatching	17	160.8	7.0	10.0	102.67		
Atlantic salmon, *Salmo salar*	eyed	10	43.14	2.17	3.1	27.36	critical O_2 level for supply of O_2 demand and non O_2-dependent metabolism	Hayes et al. (1951) (from Wickett, 1954)
	hatching	10	98.83	4.97	7.1	62.67		
Northern pike, *Esox lucius*	from fertilization to feeding larvae	15,19	>5.18–5.66	>2.16–2.35	>3.35–3.09	>33.0	necessary O_2 level for proper hatching, survival and development	Siefert et al. (1973)
Mummichog,[c] *Fundulus heteroclitus*	fertilization to hatching	20	101.2	3.15	4.5	64.9	reduced hatching at this level compared to 7.5 mg/liter O_2	Voyer and Hennekey (1972)
Pacific cod, *Gadus macrocephalus*	fertilization to hatching	3–5			>2–3		oxygen level required for optimal hatching at 15‰ salinity	Alderdice and Forrester (1971)

[a]European species — representatives of this family found in Canadian Atlantic waters.
[b]Calculations based on 4 C.
[c]Estuarine fish — calculations based on salinity of 30‰.

"incipient lethal tension." Below this level organisms resist for a time but eventually die. The point at which metabolic rate ceases to be dependent on available oxygen is termed the "incipient limiting tension." It is identical with the critical oxygen tension of Prosser and Brown (1962), termed Pc, and is frequently given in Tables 4A–D as the "no-effect oxygen threshold" that affords good protection for fish species. This relationship seems to hold for a variety of species, from fish eggs to invertebrates, and will be frequently referred to in this paper. In animals tolerant of low oxygen, the curve in Fig. 2 is pushed to the left, while in animals requiring

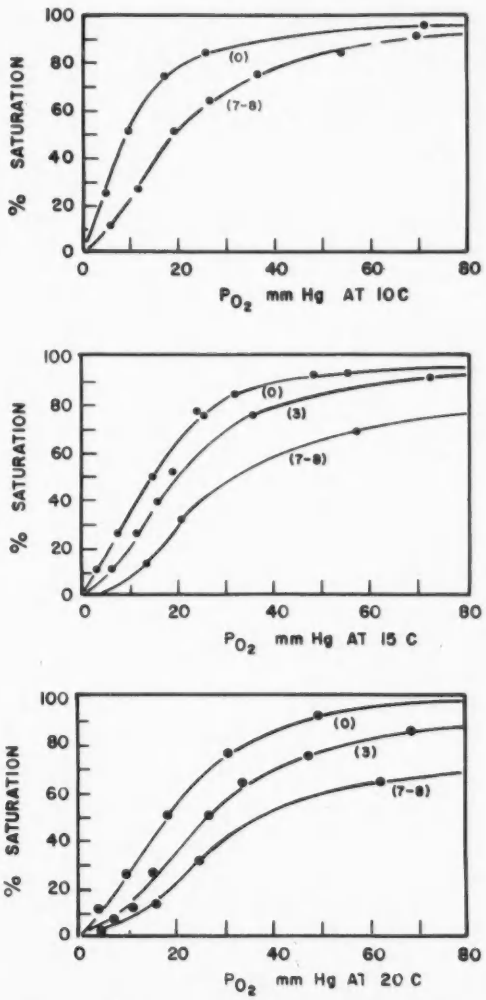

FIG. 1. Blood oxygen dissociation curves for rainbow trout (*Salmo gairdneri*) blood, plotted as O_2 tension vs. percentage oxygen saturation in the blood at three temperatures. CO_2 tensions accompanying each curve are indicated below it in parentheses (from Cameron 1971).

considerable oxygen the curve shifts right. Thus the oxygen tension that produces respiratory dependence varies with species.

Different species of teleosts (bony fish) vary in ability to regulate oxygen uptake over a range of available oxygen tensions. Elasmobranchs (cartilaginous fish) are apparently less able to regulate oxygen uptake in this way (Randall 1970). It should be remembered that levels of hypoxia severe enough to provoke the above responses occur at oxygen levels well above those acutely lethal to fish.

The responses of fish to hypoxia represent some form of compensation or adjustment of bodily processes. Fish can resist or tolerate reduced levels of oxygen for short periods. The success of tolerance depends on species, the oxygen levels, and on environmental factors, particularly temperature. Use of regulatory or compensatory mechanisms requires energy expenditure and will consequently reduce energy reserves for swimming, feeding, avoiding predators, and other activities.

ENERGETICS AND LOW OXYGEN

Considerable work has been done on the oxygen requirements of quiescent fish under laboratory conditions. Much of the earlier work on harmful or lethal oxygen levels is of this nature. Fry (1957) recognized that the respiratory dependence phenomenon should be studied in relation to active metabolism, i.e. the level of oxygen uptake accompanying maximal swimming activity. Brett (1964) studied oxygen uptake and swimming performance of young sockeye salmon and obtained evidence that available oxygen levels may limit active metabolic rate above 15 C (Fig. 3). Brett (1970) also determined the oxygen requirements for fingerling sockeye salmon at 20 C during various important activities:

Activity	O_2 Cost of energy expenditure	% active metabolic rate
feeding — max. ration	450 mg/kg per h	55.4%
feeding — maintenance ration	300 mg/kg per h	37.3
migrating up vigorous river	625 mg/kg per h	75.0
aggression	180 mg/kg per h	22.9

In light of the above data, examination of Fig. 3 indicates that a reduction in oxygen to 50% saturation would severely limit energy expenditure for migrating or maximum feeding at the higher temperatures. Fry (1957) stated:

"Any reduction of the oxygen content below the level where the active metabolic rate begins to be restricted is probably unfavorable to the species concerned. From the ecological point of view this 'incipient limiting level' (the critical level under conditions of activity) can be taken as the point where oxygen content becomes unsuitable."

In a theoretical analysis, Jones (1971a) proposed that for salmonid fish at low temperatures (5 C) the oxygen cost of the cardiac pump limits

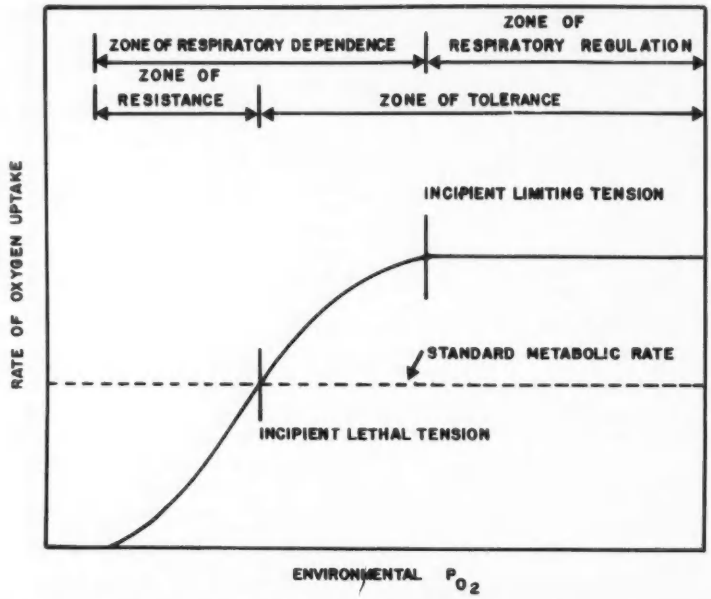

FIG. 2. Relation between standard and active (maximum) oxygen uptake rates at different environmental oxygen concentrations (from Hoar 1966).

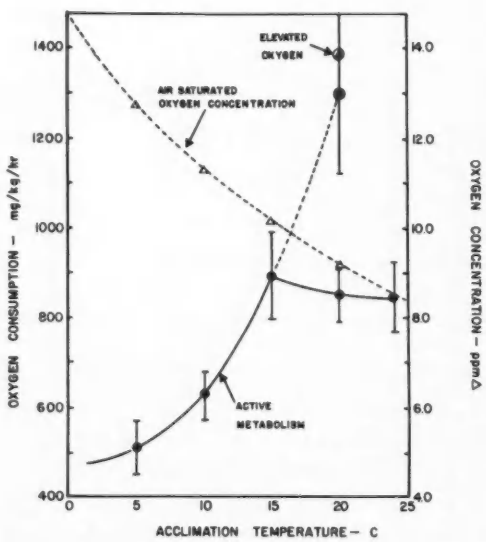

FIG. 3. Active metabolism of yearling Sockeye salmon (*Oncorhynchus nerka*) (heavy line) tested at air-saturated oxygen levels (dotted line). Mean ± 2 SE metabolic rate for elevated oxygen (13.9 ppm) determined at 20 C (circled points) (from Brett 1964).

maximal oxygen uptake during swimming; but at high temperatures (25 C) the oxygen cost of the branchial pump becomes limiting. Reductions of maximal swimming speed in rainbow trout of 30 and 43% resulted when environmental oxygen fell to 50% of saturation at 21–23 C and 8–10 C, respectively (Jones 1971b).

It should be emphasized that swimming ability in reduced oxygen may be affected by season and temperature. For example, Katz et al. (1959), reported that 6-cm largemouth bass, *Micropterus salmoides*, could resist a water current of 25 cm/s at 25 C in September even at O_2 levels of 2.0 mg/liter. However, in December at 15–17 C they were apparently unable to do so even when oxygen content was 5.0 mg/liter. In contrast, Davis et al. (1963) reported that any reduction from ambient oxygen at 10–20 C usually reduced maximum sustained swimming speed in coho and chinook salmon.

BEHAVIORAL RESPONSES TO HYPOXIA

Literature records of altered behavior of fish in low oxygen are often contradictory owing perhaps, in part, to varying experimental conditions and methods applied in studying fish

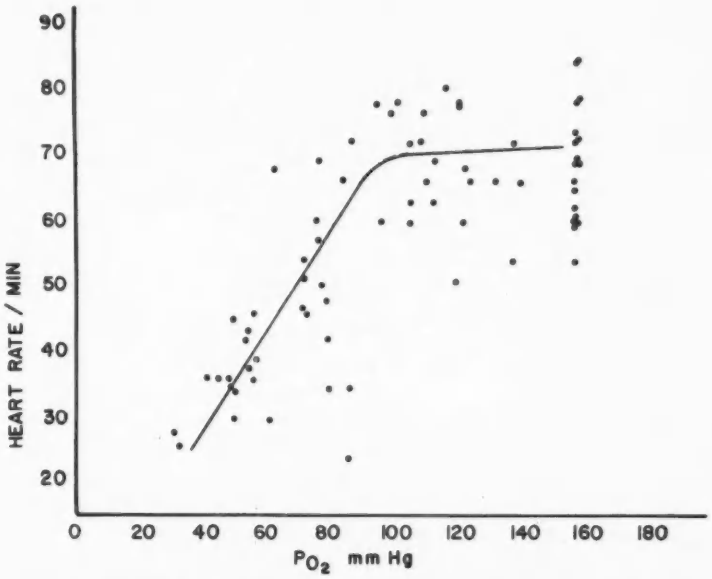

FIG. 4. Onset of bradycardia (slowing of heart beat rate) in rainbow trout in response to water oxygen tension. Solid line was fitted following a statistical analysis of the data (from Randall and Smith 1967).

behavior. It has often been stated that fish generally become more active in hypoxic water and attempt to move away from the low oxygen region (Randall 1970). Avoidance behavior has been reported for a number of species, although the reported O_2 threshold for a given response often varies considerably. For example, Whitmore et al. (1960) observed that juvenile chinook salmon (*Oncorhynchus tshawytscha*) showed marked avoidance of 1.5–4.5 mg O_2/liter in the summer at high temperatures but showed little avoidance of 4.5 mg O_2/liter in the fall. As described earlier, problems of hypoxia may develop at high temperatures where reduced water O_2 content and elevated metabolic rates would prevail. Thus a seasonal variability in behavioral sensitivity to dissolved oxygen does not seem unreasonable.

A number of other behavioral responses to hypoxia have been reported. Scherer (1971) described loss of normal avoidance of high light intensities (negative phototaxis) in walleye (*Stizostedion vitreum*) when O_2 fell to 2–4 mg/liter. Shepard (1955), studying speckled trout, *Salvelinus fontinalis*, reported "a violent burst of activity involving all the individuals in a sample" with evident "attempts to surface" when oxygen deficient water was introduced to test chambers. He further reported that fish held at O_2 levels slightly above incipient lethal levels (1.75–1.9 mg O_2/liter) "were sluggish in their movements, fed poorly and were susceptible to disease."

It is not clear whether avoidance behavior in response to low oxygen constitutes a highly directed form of behavior. It may result simply from increased locomotor activity with more random movement, which is satisfied by discovery of improved oxygen conditions. It seems likely that avoidance behavior in low oxygen would have survival value and that the presence of this behavior pattern suggests that fish periodically may benefit from it.

Implicit in a behavioral response to low oxygen would be some means of detecting low oxygen water. The physiological responses to hypoxia are rapid, consisting of circulatory and ventilatory changes in trout and tench within a few seconds of the onset of hypoxia (Eclancher 1972; Randall and Smith 1967; Fig. 4). Such rapid responses suggest the presence of an oxygen receptor system on or near the gills. The precise location of this is not yet clear. The response is rapid enough to suggest that a reflex mechanism is involved (Dejours 1973).

It is also possible that behavioral responses to low oxygen are triggered by the circulatory and ventilatory changes necessary to compensate for

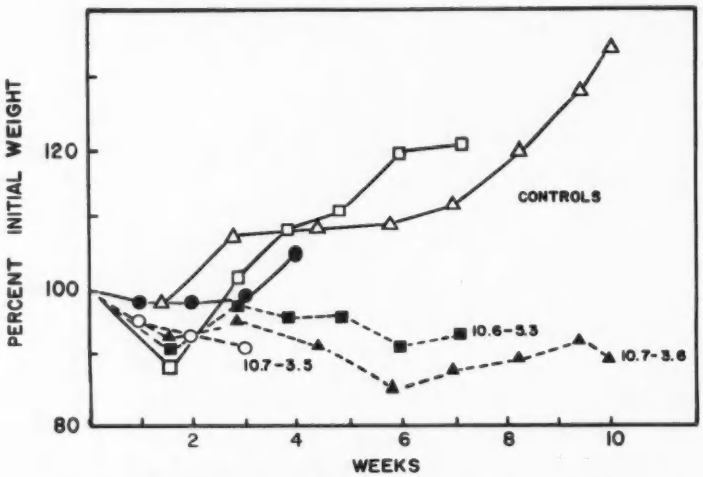

FIG. 5. Growth rate in yearling eastern brook trout (*Salvelinus fontinalis*) in constant high (solid lines — controls) and various daily fluctuating levels of oxygen (broken lines with O_2 range in mg O_2/liter) (from Fry 1970).

hypoxia. Such a mechanism would help to explain the varied behavioral thresholds reported for hypoxia, since the response might be triggered at a higher O_2 level when metabolic needs are large (for swimming, feeding, high temperatures, etc.) but not when they are minimal.

GROWTH IN REDUCED OXYGEN

High environmental oxygen levels seem to encourage growth rates in experimental fish. Stewart et al. 1967) reported that growth rate and food consumption in juvenile largemouth bass, *Micropterus salmoides*, at 26 C increased markedly as oxygen increased to near air-saturation levels and then decreased above those levels. Herrmann (1958) obtained similar results for juvenile coho salmon at 20 C as follows:

constant O_2 level — mg O_2 liter	21–27 day weight gain
4	56%
5	68%
6	88%
8	92%

He concluded that at temperatures around 20 C the "minimal oxygen concentrations to which juvenile coho salmon may be exposed for relatively long periods without markedly affecting growth, feeding, food conversion, and general activity, lie within the range of four to six milligrams per liter." Lozinov (1956) gave the optimum oxygen level for growth of young sturgeon fry as 5.0–5.5 mg O_2/liter. Davison et al. (1959) observed that juvenile coho salmon at 20 C or less in the summer or fall fed well and gained weight at 2.9 mg/liter, but concluded that their fish were "probably somewhat affected."

Exposure to cycling oxygen levels appeared to reduce growth of largemouth bass juveniles at 26 C (Stewart et al. 1967). Food conversion efficiency was reduced at oxygen concentrations below a constant O_2 level of 4.0 mg/liter. Similarly, Whitworth (1968) observed loss of weight in brook trout exposed to daily fluctuations in oxygen (Fig. 5).

ACCLIMATION TO LOW OXYGEN

There are a number of indications that fish can acclimate, to some degree, to lowered oxygen levels. Shepard (1955) showed that the lethal O_2 level for brook trout, *Salvelinus fontinalis*, could be reduced with low oxygen acclimation. He suggested that adjustments might be made in the oxygen carrying capacity of the blood, thus increasing the ability to extract O_2 from the water. MacLeod and Smith (1966) showed that fathead minnows, *Pimephales promelas*, increased hematocrit (packed volume of red blood cells) in response to low oxygen, thus making more hemoglobin available for O_2 transport in the blood. However, Cameron (1970), in assessing hematological responses of pinfish, *Lagodon rhomboides*,

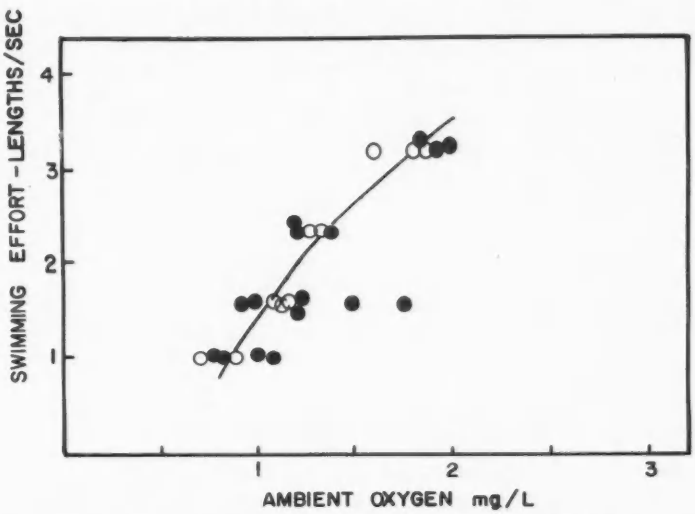

FIG. 6. Swimming ability vs. oxygen availability in goldfish (*Carassius auratus*). Open circles denote fish acclimated to air-saturation O_2 levels; closed circles indicate fish acclimated to 15% oxygen saturation. Acclimation to low oxygen did not appear to enhance swimming ability in low oxygen water under these conditions (from Kutty 1968).

and striped mullet, *Mugil cephalus*, concluded that changes in blood oxygen capacity induced by low oxygen acclimation were small and of slight significance to respiration, rather than the extensive compensatory hemoglobin production previously proposed for goldfish (Prosser et al. 1957).

As well as resulting in blood oxygen capacity changes, acclimation could be behavioral in nature. It is possible that considerable energy could be expended by a nonacclimated fish reacting behaviorally to low oxygen conditions. Acclimation could lower the magnitude of these reactions and might thus enable conservation of energy under oxygen stress.

Acclimation that enhances survival at near-lethal conditions may not always aid active processes. Kutty (1968b) found no difference in swimming speed at limiting oxygen levels in goldfish acclimated to both low and air-saturation oxygen levels (Fig. 6). In addition, he observed that reduced oxygen consumption existed in low O_2-adapted fish at a given swimming speed compared to fish held at high oxygen levels.

There is a scarcity of field data to show that ability to acclimate to low O_2 markedly improves survival chances of fish populations. Any such ability to adapt to low oxygen should be useful to some degree, especially if the transition to a low oxygen regime is slow enough to enable acclimation to occur without severe physiological stress. However, the mechanism would be of little help to fish suddenly encountering low-oxygen water. Acclimation ability can be considered as a built-in safety factor for some species encountering low oxygen conditions. I consider that there is insufficient evidence to warrant modification of oxygen criteria to include acclimatory ability of fish.

INTERACTION OF LOW OXYGEN AND TOXIC AGENTS

Areas polluted with industrial waste, sewage, and other contaminants frequently have both a low oxygen and a toxicity problem. Often a number of different wastes are discharged into a body of water from various sources. The effects of these discharges can be additive. Alderdice and Brett (1957) observed an apparent increase in toxicity of kraft pulpmill waste to young sockeye salmon in the presence of low oxygen. Townsend and Cheyne (1944) reported a similar toxic interaction of low oxygen and elevated hydrogen ion concentrations to coho salmon. Markedly increased toxicity of ammonia solutions, salts of zinc, lead, and copper and a mixture of monohydric phenols, was evident in

rainbow trout when oxygen levels fell below 60% saturation at 17.5 C (Lloyd 1961). Lloyd felt that toxicant delivery to the gills, and its rate of uptake, was enhanced by the increased respiratory flow necessary to counteract hypoxic conditions. Presumably high water temperatures, hyperactivity, or some other metabolism-stimulating condition elevating respiratory activity, would produce a similar result.

Egg and Larval Development in Low Oxygen

There are several studies on the dissolved oxygen requirements of developing fish eggs and larvae. Like fish, eggs also show respiratory dependence (Fry 1957) (Fig. 7). Alderdice et al. (1957) reported a gradual increase in the dissolved oxygen requirements of chum salmon eggs, *Oncorhynchus keta*, as development progressed. Eggs at early stages of incubation required about 1 mg O_2/liter, while those about to hatch required over 7 mg O_2/liter. Eggs held at low oxygen tensions showed reduced growth and retarded development. Deformities in embryos exposed to hypoxia were observed, with mortality occurring at the stage when the circulatory system develops. Other workers report similar increased oxygen requirements of eggs as development proceeds (Guilidov 1969; Hayes 1949; Lindroth 1942; Wickett 1954).

Larval fish, like eggs, are affected by reduced oxygen. The oxygen demand of larval fish increases markedly with age (Sharmardina 1954). Nikiforov (1952) reported complete yolk sac absorption and better growth in 5-day-old *Salmo salar* larvae reared for 25 days at 6.8–7.5 mg O_2/liter, compared to those reared at 4.5–5.0 mg O_2/liter. The latter group weighed less than one half of the high-oxygen reared group and had one third of the yolk sac unabsorbed.

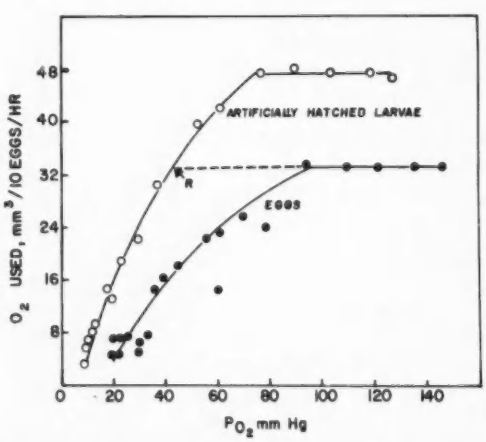

FIG. 7. Oxygen uptake rates in eggs and larvae of Atlantic salmon, *Salmo salar*, at 10 C, illustrating respiratory dependence and the influence of the egg capsule (from Fry 1957).

Circulatory and ventilatory responses of fish larvae to low oxygen have been described. Holeton (1971) observed that oxygen tensions below 100 mm Hg stimulated heart rate and breathing rate in 1-day-old rainbow trout larvae. Similar, but more marked responses, were seen in 8-day-old larvae. In these older larvae, PO_2 levels of 45–50 mm Hg produced reduced breathing rate and increased depth (amplitude) of breathing.

There is very little information on the dissolved oxygen requirements of marine fish eggs. It seems likely that marine eggs, when close to hatching, require fairly high oxygen levels similar to eggs of freshwater species. Conceivably, an oxygen level acceptable to adult marine fish may be unsuitable for marine eggs — a factor that could easily be overlooked!

Summary of Oxygen Requirements of Fish

Reduction in the level of available oxygen has a marked effect on many physiological, biochemical, and behavioral processes in fish. The oxygen level where such effects first become apparent — i.e. the incipient oxygen response threshold, has been used throughout this report in the derivation of oxygen criteria selected to assess adverse effects on fish.

Restriction in the supply of oxygen available for metabolic processes including swimming, migrating, and feeding are likely caused by hypoxia. Adequate oxygen levels for such activities are necessary for survival of healthy fish populations. Oxygen requirements of fish are noticeably influenced by season, temperature, and activity. The oxygen tension where respiratory dependence begins is considered an important lower limit for tolerable available oxygen. Effects of hypoxia on metabolic processes and swimming ability, as well as O_2 levels causing the onset of respiratory dependence, are thus considered good indicators of the incipient sublethal effect threshold for low oxygen.

The blood oxygen dissociation curve delineates the water oxygen tension where the blood ceases to be fully oxygen saturated. For optimal gas exchange, water PO_2's, preferably 20 mm Hg above that saturation point are required in trout to maintain the necessary oxygen gradient at the gills. Oxygen dissociation curves, therefore, provide an indication of the point where available

oxygen limits or influences respiratory and circulatory functions. The shape of the blood curve and position of the limiting O_2 tension are influenced by temperature and CO_2, such that increases in these factors elevate the PO_2 required to fully saturate the blood. Limiting oxygen tensions, therefore, can be derived from blood curves and are useful for defining low oxygen effects on fish. In addition, oxygen response thresholds for circulatory and ventilatory systems have been described.

Low oxygen levels have been shown to influence growth rate, food conversion efficiency, and feeding in some species. In some cases, cycling oxygen levels appear to adversely affect growth under laboratory conditions. As growth is an expression of the net product of metabolic functions, it is a good indicator of an overall effect of low oxygen. Therefore, adverse effects of low O_2 on growth constitute a useful criterion for permissibly low oxygen levels.

Avoidance behavior has been reported for a number of species in response to low oxygen, although reports of O_2 thresholds to initiate this behavior vary considerably. Observed behavior patterns in low O_2 include increased activity, poor feeding behavior, and altered phototaxis. Avoidance behavior is likely a useful protective mechanism that enhances the survival of some species.

It is apparent that low oxygen in the presence of some toxicants enhances the lethal effect of those toxicants on some fish. Low oxygen levels producing metabolic stress could lower the ability of fish to resist toxicants as well as increase toxicant uptake rate via elevated ventilatory water flow. For these reasons the need for sound scientific dissolved oxygen criteria should be obvious, especially in geographical areas polluted by discharges toxic to fish.

Some fish show ability to acclimate to lowered ambient oxygen. However, the degree of advantage gained is not clear. Acclimation can enhance blood oxygen capacity and oxygen utilization somewhat but may not aid active swimming performance. This acclimation phenomenon may be useful to fish subjected to gradual reductions in oxygen levels, but would be of little advantage when encountering reduced oxygen levels for the first time, especially if the O_2 depression was severe. For these reasons, the application of acclimation phenomena to water oxygen criteria for fish protection has not been attempted in a subsequent section of this report and can be considered only as a possible safety factor enhancing the fish's resistance to low oxygen.

Developing fish eggs and larvae show a number of responses to low oxygen including respiratory dependence, retarded growth, reduced yolk sac absorption, developmental deformities, and mortality. As development proceeds, the oxygen requirements of both eggs and larvae increase. Trout larvae exhibit circulatory and respiratory effects of low oxygen. Most data on the effect of low oxygen on fish eggs and larvae are confined to freshwater species, particularly salmonids, and there is little informatiton for marine eggs and larvae.

It is particularly evident that much of our knowledge of low O_2 effects on fish tends to be confined to freshwater species, particularly salmonids. There is a great lack of knowledge available on the oxygen requirements and tolerances of nonsalmonid species and their eggs and larvae. The need for more research in this area is strongly emphasized.

Oxygen Requirements of Aquatic Invertebrates

There is extensive literature pertaining to measurement of metabolic rate (oxygen uptake) of aquatic invertebrates under various environmental conditions such as temperature, salinity, pH, CO_2 tension, etc. Indeed, a considerable body of knowledge is available on the interaction of environmental oxygen availability, oxygen uptake, and ventilation rates for many freshwater, marine, and estuarine organisms. The following is an attempt to summarize some of the general findings of this rather extensive literature. Unlike the fish section of this report, this section is not mainly confined to a discussion of purely Canadian species. Wherever possible, however, attention is given to reporting results from work with Canadian species. In my opinion, knowledge of invertebrate community oxygen requirements is too incomplete to warrant the setting of definite "ecologically safe" oxygen criteria for invertebrate communities. The following discussion was designed to review current general knowledge and attempts to arrive at some general recommendations that can be followed by those attempting to define safe oxygen levels for prolonged survival of invertebrate communities.

Aerobic and Anaerobic Metabolism

Most aquatic organisms can utilize several metabolic pathways as a means of obtaining energy. These processes can be aerobic (utilizing oxygen) or anaerobic. Anaerobic processes yield energy in the absence of oxygen. An excellent discussion of the various metabolic pathways, their evolution, and relationship to oxygen availability can be found in Hochachka and Somero (1973).

TABLE 5. Summary of A) oxygen independent (regulated O_2 uptake) and B) oxygen dependent (O_2 conforming) groups of organisms (from Prosser and Brown 1962).

A) Oxygen independent
Most protozoa — *Tetrahymera*, *Paramecium*, *Trypanosoma*
Coelenterates — *Hydra* between 10 and 100%. O_2 in the gase phase
Annelids — *Tubifex*, *Lumbricus*, the leeches *Glossiphonia* and *Hirudo*, larvae of some polychaetes, Pc = 40 to 50 mm
Echinoderm eggs — *Arbacia*, unfertilized, Pc = 40; fertilized, Pc = 50 mm Hg
A few parasitic worms — *Trichinella*, Pc = 8 mm; *Nematodirus*, *Haemonchus*, *Rhabditis elegans*, Pc = 121 mm; *Rhabditis strongloides*, Pc = 58.5 mm
Molluscs — clam *Mya*, Pc = 40 to 50 mm; oyster, Pc = 100 mm; snail *Ancylus*, Pc = 80 mm; snail *Australabrus*, Pc = 30 mm; *Mytilus*, Pc = 50% air saturation
Crustacea — copepod *Calanus*, Pc = 204 ml O_2/liter; crayfish, Pc = 40 mm; crab *Pugettia*, Pc = 70 mm; *Uca pugilator* and *Uca pugnax*, Pc = 4 mm
Insects — termite *termopsis*, Pc = 38 mm; diapausing cecropia pupae, Pc = 23 to 28 mm; several chironomid larvae, Pc = 55 to 68 mm
Most aquatic vertebrates, Pc = 30 mm Hg
Crucian carp — 30 mm Hg at 5 C
All terrestrial vertebrates; probably all terrestrial insects
B) Oxygen dependent
Protozoa — *Spirostomum*
Numerous coelenterates — actinians, *Cassiopea*
Free-living worms — *Sipunculus*, *Urechis*, leeches *Piscicola* and *Erpobdella*, *Nereis*
Most parastic worms — trematodes — *Fasciola*, *Schistosoma*, cestode *Diphyllobothrium*, nematodes *Ascaris*, *Litomosoides*, *Limax*, strongyles larvae
Some molluscs and crustaceans — *Limes*, *Limulus*, *Homarus*, series of marine crustaceans daphnid *Simocephalus* fifth instar conforms over environmental range 14.6 to 1.1 cm^3 O_2/liter
Echinoderm adults — starfish, sea urchins
Some aquatic insects — *Ephemera*, *Anatopynia*, *Tanytarsus*
Few vertebrates — toadfish, *Triturus pyrrhogaster*, cutaneous respiration

The degree to which organisms can utilize anaerobic processes varies considerably and thus determines the tolerance of individual species to low oxygen conditions. Anaerobiosis is common in parasites and some, such as the intestinal flagellates of termites, may be poisoned by small amounts of oxygen (Prosser and Brown 1962). Others such as mud-dwelling tubificid worms can live for long periods in water nearly devoid of oxygen. Many organisms are normally aerobic but can survive many hours without oxygen (e.g. frogs, earthworms, cockroaches, aquatic snails) by utilizing anaerobic means of energy production. In contrast, many aerobic animals are highly dependent on oxygen. Typically, these animals can build up only a brief oxygen debt (accumulated metabolic products of anaerobic metabolism, such as lactic acid, or other compounds, which must be later oxidized), particularly in their muscles. Examples of these organisms are birds, mammals, many adult insects, and cephalopod molluscs (Prosser and Brown 1962). In general, animals with high metabolic rates are less tolerant of low O_2 than sluggish forms; early eggs and cysts and small organisms are often tolerant of low oxygen, as are hibernating or behaviorally-inactive organisms.

It should be emphasized that anaerobic processes yield less energy per unit glucose metabolized compared to aerobic processes (Lehninger 1965). The utilization of anaerobic pathways is therefore accompanied by some reduction of available energy. This is likely not a serious consequence for small, relatively inactive species but would be problematical to larger, active species. A reduction in available oxygen would therefore most seriously affect the more active, high-energy utilizing members of a community. These generalizations will be discussed in the following sections on animals inhabiting different environments.

RESPIRATORY DEPENDENCE IN INVERTEBRATES

Earlier it was pointed out that the phenomenon of respiratory dependence (a correspondence of the rate of oxygen uptake with available oxygen level) occurs in many fish as well as in their eggs and larvae. Often there is a critical oxygen tension for invertebrates as well (Pc — Prosser and Brown 1962) below which respiratory dependence begins. Prosser and Brown describe organisms whose oxygen uptake rate is independent of available O_2 level above the Pc level (Table 5A) and

others (Table 5B) whose O_2 uptake rate conforms to the available O_2 level.

It can be seen from Table 5 that many aquatic invertebrates possess the ability to regulate oxygen uptake over a range of oxygen tensions, while many others show conformity (dependence). Furthermore, the level of Pc varies considerably, sometimes even between closely related forms. Nicol (1967) suggested that a number of factors determine whether an animal is dependent or independent of the environmental oxygen level. These factors include size, existence of a circulatory system, diffusion distances, temperature, degree of locomotor activity, ability to regulate external respiration, and the existence of respiratory pigments.

Examples of respiratory dependence and independence will be discussed in the following sections.

Oxygen Requirements of Some Freshwater Invertebrates

The oxygen-sensitivity and oxygen uptake rates of many freshwater organisms appear to reflect the habitat in which they live. For example, Fox et al. (1937) reported the following results with mayfly nymphs:

Genus	Habitat	O_2 uptake at air satn. (mgO_2/g/h)	Pc (ml O_2/liter)	Pc (mg O_2/liter)
Baetis	swift streams	2.8	12.0	17.00
Leptophlebia	lake	2.1	2.5	3.60
Cloean	pond	1.3	2.0	2.86

Von Brand et al. (1950) reported that Lymnaeidae and Physidae groups of freshwater snails could withstand 6 h of anaerobiosis, while Planorbidae and Operculate snails survived at least 24 h. *Donacia* larvae (Chrysomelidae: Coleoptera) survived 168 h in closed bottles that had only 0.26 ml O_2/liter (0.37 mg O_2/liter) at the start of the test (Hoffman 1940). Also, Cole (1926) reported that freshwater clams, *Anadontoides ferrussacianus*, could live for several days in the absence of oxygen. These species inhabit muddy bottoms where oxygen may be periodically low. Sprague (1963) observed 50% mortality in 24 h in the isopod *Asellus intermedius* at 0.03 mg O_2/liter, and mortality thresholds of 0.7, 22, and 4.3 mg O_2/liter for the amphipods, *Hyalella azteca*, *Gammarus pseudolimnaeus*, and *Gammarus fasciatus*, respectively. Of these, *A. intermedius* is often found in polluted and deoxygenated water, whereas records of *G. fasciatus* inhabiting deoxygenated water are scarce.

Moshiri et al. (1970) reported dependent respiration in the crayfish, *Pacifastacus leniusculus*. At 20 C over a range of 2–7 ml O_2/liter oxygen utilization was elevated below 4.0 ml O_2/liter (=5.7 mg/liter) and ventilation depressed below 2.5 ml O_2/liter (=3.6 mg/liter). Larimer and Gold (1961) found similar respiratory dependence in the crayfish, *Procambarus simulaus*, and a stimulation of ventilation when oxygen levels dropped. Hoglund (1961) showed that the crayfish, *Astacus fluviatilus*, responded to oxygen gradients by moving from low to high O_2 concentration. Berg (1952) compared related freshwater snails, *Ancylus fluviatilus*, and *Acroloxus lacustris*, and noted that *Acroloxus* could survive much longer anaerobic exposure than *Ancylus*. Berg suggested this difference could be related to *Acroloxus*' preference for stagnant conditions.

Various responses of freshwater organisms to low or declining oxygen levels have been described. Chironomid larvae and the leech, *Erpobdella testacea*, are able to regulate oxygen uptake and lower their Pc following acclimation to low oxygen. *Daphnia* produce hemoglobin and turn red after stimulation with low oxygen for about 10 days (Prosser and Brown 1962). *Donacia* larvae become inactive in low oxygen water and resume activity when oxygen levels rise (Hoffman 1940). *Daphnia magna* show a preference for fully saturated water over that only 15% saturated, although more than an hour is required for the response to develop (Fig. 8) (Ganning and Wulff 1966). *Gammarus pulex* (Costa 1967) increases its pleopod movement in low oxygen and moves out of low oxygen water. A slowly developing avoidance reaction to oxygen contents up to 7 mg O_2/liter was demonstrated (Fig. 9). Freshwater snails, *Ancylus fluviatilus*, climb to-

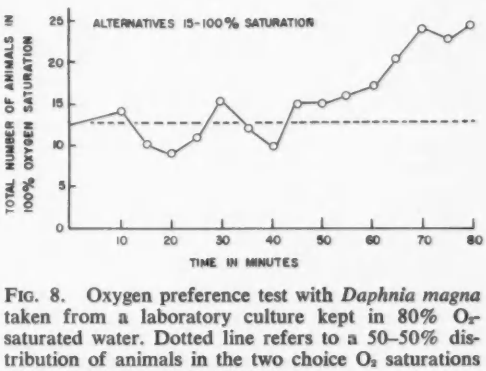

Fig. 8. Oxygen preference test with *Daphnia magna* taken from a laboratory culture kept in 80% O_2-saturated water. Dotted line refers to a 50–50% distribution of animals in the two choice O_2 saturations (from Ganning and Wulff 1966).

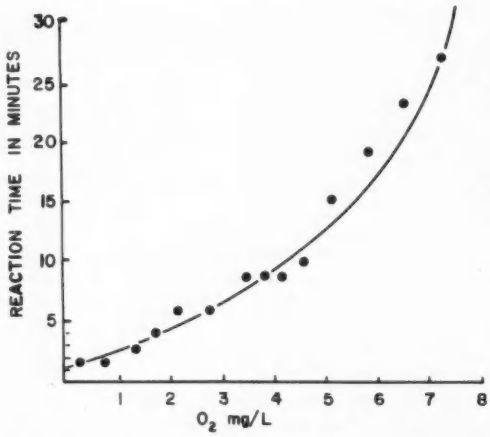

FIG. 9. Time for the onset of avoidance behavior to low oxygen water by *Gammarus pulex* at 14–15 C (from Costa 1967).

wards the surface of the water when dissolved oxygen drops (Berg 1952). Chironomids suspend feeding when oxygen drops to 5–7.5% of saturation (Prosser and Brown 1962).

OXYGEN REQUIREMENTS OF SOME INTERTIDAL MARINE INVERTEBRATES

During the night at low tide, oxygen levels in pools and animal burrows may drop substantially, depending on community respiration rates, population density, organic decomposition, and other factors. The lugworm, *Arenicola marina*, cannot tolerate oxygen in water above 4% of saturation. Fully O_2-saturated water is slowly toxic, reflecting this species' tolerance and specialization for periodic anoxic conditions. In contrast, *Sabella pavonina*, a tubicolus polychaete normally dwelling in well-aerated water, tolerates 10% O_2 saturation but dies in 4 days at 4% (Nicol 1967). The polychaete *Nereis diversicolor* reduces its rate of water pumping in low O_2, as does *Arenicola*, possibly to conserve energy. *Gammarus oceanicus* from Nova Scotia tide pools avoid experimental anoxic conditions and become more active (Cook and Boyd 1965). Oysters, *Ostrea virginica*, survive exposure for at least a week to water containing less than 0.5 mg O_2/liter (Mitchell 1912). Similarly, the mussel, *Mytilus edulis*, can survive several weeks without oxygen (Moore 1964).

Nematodes and mites, inhabiting large coastal sea weeds (*Fucus*, *Ascophyllum*) which collapse at low tide and consume oxygen from tide pools, can survive anaerobic conditions for 16 h at 25 C (Wieser and Kanwisher 1959). Marsh crabs, *Uca pugnax* and *U. pugilator*, show respiratory independence down to a PO_2 of 40–50 mm Hg, and can withstand anaerobic conditions for at least 1 wk (Teal and Carey 1967).

Pacific sea urchins provide a good example of utilization of various degrees and modes of respiration related to O_2 availability and demands of metabolism. *Strongylocentrotus drobachiensis*, *S. franciscanus*, and *S. purpuratus* show respiratory dependence below 60–70 mm Hg PO_2 (Fig. 10), and below that level, oxygen tensions in water and coelomic fluid show close correspondence (Fig. 11). Indeed, these urchins appear able to reduce their internal coelomic fluid PO_2 even in well-aerated water, possibly utilizing energy-saving mechanisms in doing so (Johansen and Vadas 1967). Furthermore, urchins can survive up to 15 h exposure to moist air. The gas exchange occurring under these conditions is enough to support an oxygen uptake rate ½–⅓ of the maximum rate observed in aerated water.

Some amphipods, frequently found under rocks, logs, and algae on Pacific shores can tolerate low oxygen. Waldichuk and Bousfield (1962) observed numerous *Allorchestes angustus* and some *Anisogammarus pugettensis* in surface water containing 0.3–0.89 mg O_2/liter (near a Sulphite pulp mill). C. D. Levings (unpublished cruise report — Ocean Falls Harbour, B.C.) lowered baskets of *Anisogammarus confervicolus* into a low oxygen layer 40 m deep. All the amphipods survived 24 h exposure to 0.52–1.25 ml O_2/liter (0.74–1.8 mg O_2/liter) but died after 36 h.

OXYGEN REQUIREMENTS OF BENTHIC MARINE ORGANISMS AND COMMUNITIES

Marine benthos are often exposed to low oxygen conditions due to oxidative processes occurring in or near the sediments that deplete water of oxygen. The severity of the depletion depends upon many factors including geographical position, depth, tides and currents, population structure, settlement rate of organic matter, and aerobic respiration of organisms.

In many instances the oxygen level most suitable for benthic dwellers can be related to their life habits. The amphipod *Corophium arenarium* inhabits aerobic sediments, while *Corophium volutator* is found in anaerobic sediments. Behavior experiments showed that *C. arenarium* has a significant preference for oxygenated water, while *C. volutator* is indifferent (Gamble 1971).

Numerous responses of benthic invertebrates to declining oxygen levels have been reported. The octopus, *Octopus dofleini*, exhibits lowered arterial blood oxygen levels and elevated cardiac output when O_2 partial pressure falls below 120 mm Hg

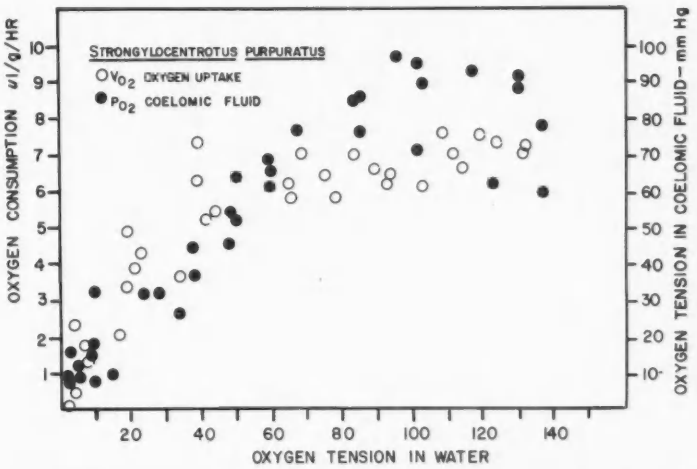
STRONGYLOCENTROTUS PURPURATUS
$O_{V_{O_2}}$ OXYGEN UPTAKE
● P_{O_2} COELOMIC FLUID
OXYGEN CONSUMPTION ml/g/HR
OXYGEN TENSION IN COELOMIC FLUID – mm Hg
OXYGEN TENSION IN WATER

FIG. 10. Oxygen uptake (left ordinate, open circles) and coelomic fluid PO_2 (right ordinate, closed circles) vs. partial pressure of ambient water in the Pacific sea urchin *Strongylocentrotus purpuratus* (from Johansen and Vadas 1967).

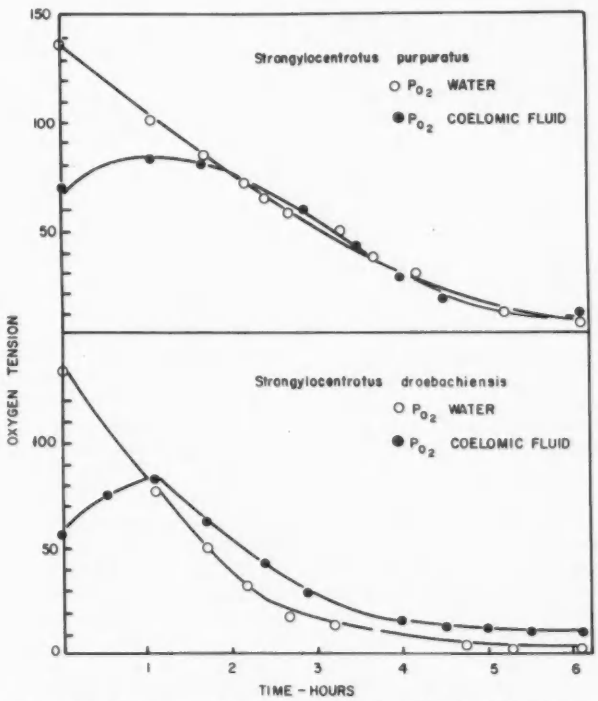

FIG. 11. Oxygen tension in water and coelomic fluid to two species of Pacific urchins observed when the urchins were in a closed respiration chamber (from Johnsen and Vadas 1967).

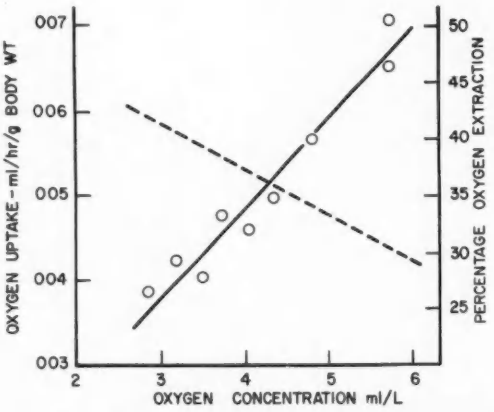

FIG. 12. Effect of water oxygen content on oxygen uptake, and percentage oxygen extracted by the gills of a 332-g European lobster, *Homarus vulgaris*. Solid line depicts oxygen uptake and the dotted line denotes percentage extraction (from Thomas 1954).

PO_2 (Johansen and Lenfant 1966). Some tendency for oxygen-dependent respiration is evident in the lobster, *Homarus vulgaris* (Fig. 12) and crab, *Cancer productus*, especially at high temperatures when oxygen availability falls (Belman and Childress 1973). Burrowing activity in wood of three marine isopods, *Limnoria lignorum*, *L. quadripunctata*, and *L. tripunctata*, (Fig. 13) appears directly related to available oxygen tension (Anderson and Reish 1967). Twenty-eight days MTL's for survival of these three species tested at three temperatures range from 0.6 to 1.18 mg O_2/liter. The calcareous tube-building polychaete, *Hydroides norvegica*, may show reduced larval settlement in low oxygen conditions (Reish 1961) as well as in low temperatures. Estuarine shrimps, *Crangon vulgaris*, are asphyxiated below 20% air saturation (21 C, 23‰ salinity), and are stimulated to swim vertically at dissolved oxygen levels between 22 and 35% of saturation (Huddard and Arthur 1971). The authors suggest that this vertical swimming response may allow the shrimps to be horizontally displaced by currents and so reach areas of improved oxygen conditions where they passively sink to the bottom. The anemonae *Actinia* moves to the surface when O_2 falls below 2 ml O_2/liter (2.8 mg O_2/liter), but if this movement is prevented it closes up and "enters a latent period of life," (Nicol 1967).

Ventilatory activity is often affected by O_2 level. *Arenicola* and *Nereis diversicolor* reduce their rate of water pumping in burrows, while another polychaete, *Chaetopterus variopedatus*, increases its pumping rate. The lobster *Homarus*

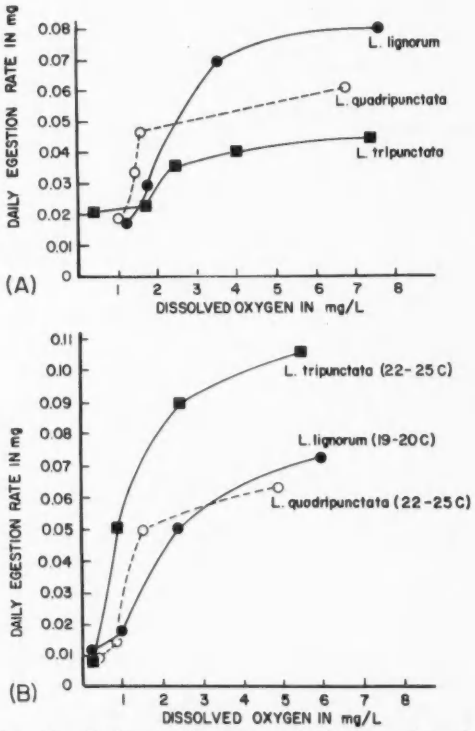

FIG. 13. Daily egestion rates of three species of *Limnoria* at different dissolved oxygen concentrations at A) 15 C and B) the temperatures indicated (from Anderson and Reish 1967).

vulgaris shows increased oxygen utilization and respiratory dependence at 15 C, in response to declining oxygen levels. Atlantic lobster kills are suspected to be sometimes related to low dissolved oxygen (Young 1973), although McLeese (1956) reported survival of American lobsters for 48 h in 1.1 mg/liter of oxygen. Crustacea often show elevated respiratory movements in low oxygen, as seen in the stomatopod, *Squilla mantis*, the prawn, *Pandalus borealis* and the crab *Carcinus maenas* (Johnston 1936).

The respiratory dependence phenomenon, exhibited by many invertebrates, can be demonstrated for benthic communities. The log of sediment oxygen uptake from a variety of benthic communities appears correlated significantly with temperature (Fig. 14A). Thus, in terms of their oxygen requirements, benthic communities of diverse geographical location appear similar. When oxygen levels fall below 6.0 mg O_2/liter, the rate of sediment-community O_2 uptake may decline (Fig. 14B). The sediment community appears to

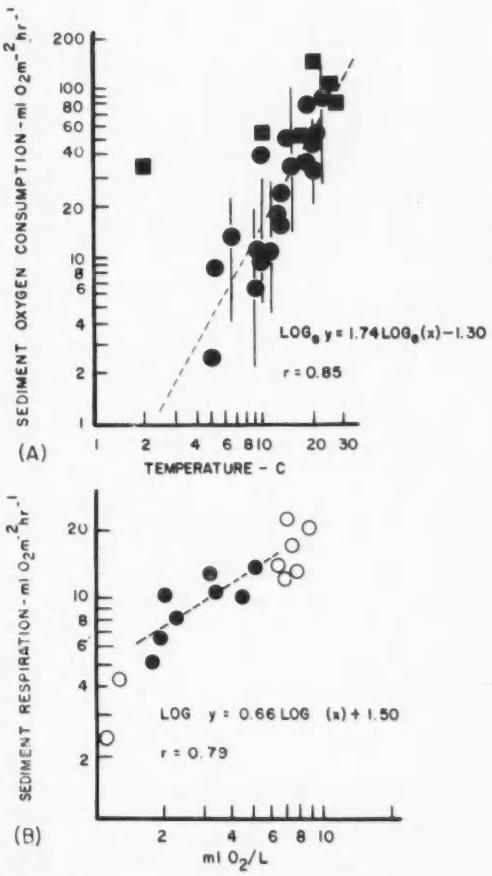

FIG. 14. A, Oxygen uptake by a variety of benthic communities, both marine and fresh water, in relation to temperature. Values are given as individual points or as ranges if the data permit, each point or range signifying a different study; B, Relationship between the sediment oxygen uptake rate and the oxygen content of overlying water at 15 C in Marion Lake, B.C. Open circles are not included in the regression calculation (from Hargrave 1969).

exhibit the respiratory dependence phenomenon, likely because of the response of the individuals composing the community. It follows that the degree of hypoxia tolerated by a community will depend on the ability of individuals within the community to withstand low oxygen. Thus, if a prolonged low oxygen regime is imposed on a community, the tolerant members will survive while the less resistant will die off and may be replaced by more tolerant forms from within or outside the community.

A comprehensive documentation of the effect of periodic anaerobic conditions on benthic community structure was made by Hoos (1973). Her study dealt with Petpeswick Inlet, Nova Scotia, where the deeper water becomes anoxic in late summer and fall. Hoos found that species diversity of benthos decreased with depth, and varied seasonally according to the available dissolved oxygen level. Below 15 m, *Nephtys incisa*, *Mytilus edulis*, *Mya arenaria*, *Capitella capitata*, and copepods were the dominant organisms when sufficient oxygen was present to support life. In the deep basin, species diversity declined to zero in September–November, 1971. When basin turnover occurred, the major recolonizers included adult *Yoldia limatuloides*, *Nephtys incisa*, *Polydora quadrilobata*, *Corophium* sp., phyllodocid polychaetes, and nematode worms. Hoos reports that her findings are similar to those of several authors, working elsewhere, who listed organisms best able to survive periods of deoxygenation that included *Nephys* sp., *Nereis* sp., *Scoloplos* sp., *Capitella capitata*, *Mytilus edulis*, *Mya arenaria*, *Corophium volutator*, harpacticoid copepods, and nematodes. Similar studies and reports of sensitivity of benthos to low oxygen in other parts of the world were made by Emery and Hulsemann (1962), Heegaard (1944), Rhoads and Morse (1971), and Tulkki (1965).

Substantial benthic communities often exist under low oxygen conditions. For example, C. D. Levings (unpublished data) examined the oxygen content and community structure below 150 m depth in two areas in the north end of Howe Sound near Vancouver, B.C. Seasonal oxygen levels at these two sample areas taken 5 m above the bottom were:

	Basin station	Woodfibre station
Date	(x, range, *n*) ml O_2/liter	(x, range, *n*) ml O_2/liter
June 1972	2.24, 2.10–2.57, 4	2.25, 1.87–2.63, 2
Sept. 1972	5.65, 4.08–7.16, 3	5.86, (identical), 2
Nov. 1973	2.79, 2.32–3.11, 4	2.54, 2.22–2.86, 2
Jan. 1973	3.03, 2.30–3.32, 7	2.42, 2.41–2.43, 2
Feb. 1973	2.84, 2.71–2.93, 4	2.71, 2.57–2.86, 2

Summaries of the polychaete worm species retained on a 0.5-mm sieve at each station are presented in Table 6. It can be seen that a variety of taxa exist in deep water where only 2–3 ml O_2/liter (2.9–4.3 mg/liter) is commonly found. The large differences in number of taxa between the two communities may be related to the presence of wood waste and/or chemical contamination at Woodfibre station, or other factors, as yet unknown.

A thriving mixed invertebrate community was described by Levings and McDaniel (1974) who examined benthos attached to an undersea cable spanning the Strait of Georgia, B.C. The cable was retrieved from deep water where the oxygen levels are between 2 and 4 ml O_2/liter (2.9 and 5.7 mg/liter). Levings and McDaniel observed "a wide variety of epifaunal invertebrates including sponges, anemones, gorgonians, polychaetes, and starfish attached to the cable retrieved from water 300–350 m deep."

Oxygen Tolerances and Requirements of Marine Planktonic and Pelagic Organisms

Organisms that swim freely in the sea, float, or are passively transported in some way, could encounter a variety of oxygen conditions. Generally, surface waters are well oxygenated while deeper layers tend to be lower in oxygen content, although there are numerous exceptions due to upwelling, river outflows, decomposition, respiration, photosynthesis, and pollution influences.

The oxygen requirements, oxygen-related behavior and physiology of pelagic fish have been reviewed previously. Pelagic invertebrates in many instances show some similar responses. The squid *Loligo* is reported to avoid oxygen-deficient water (Prosser and Brown 1962), and observations of other planktonic species' availability in low oxygen conditions are recorded. For example, Hoos (1970) observed a variety of species inhabiting an oxygen-minimum deep layer (approximately 0.4 ml O_2/liter = .57 mg/liter) in a B.C. inlet (Table 7). Hoos found that some vertically migrating species such as *Euphasia pacifica* did not enter the oxygen-minimum layer, while others such as *Calanus plumchrus* tended to remain there. In contrast, Marshall et al. (1935) concluded:

"*Calanus* are unaffected by an increase in the oxygen content of the water, but are sensitive to low oxygen tensions. Below a concentration of about 3 ml/l the respiration decreases. At concentrations between 1 & 2 ml/l they are killed. They are more resistant at 5°C than 15° and stage V are more resistant than adults at both these temperatures." (Fig. 15)

TABLE 6. Polychaete taxa retained on a 0.5-mm seive from benthic samples taken at two Howe Sound, B.C. stations in 1972 by C. D. Levings (unpublished data).

Taxa at basin stations	Taxa at woodfiber stations
Polychaeta (Families)	Polychaeta (Families)
Ampharetidae	Capitellidae
Capitellidae	Cirratulidae
Cirratulidae	Nereidae
Flabelligeridae	Nephytidae
Glyceridae	Paraonidae
Lumbrineridae	
Maldanidae	Σ 5 taxa
Nephytidae	
Nereidae	
Opheliidae	
Oweniidae	
Paraonidae	
Pectinariidae	
Phyllodocidae	
Polynoidae	
Sabellidae	
Spoinidae	
Syllidae	
Sternaspidae	
Terebellidae	
Σ 20 Taxa	

Summary of Oxygen Requirements of Aquatic Invertebrates

From the foregoing discussion it is obvious that a great range of tolerances, responses, and requirements for oxygen exist amongst aquatic invertebrates. Separate aquatic communities are also likely to differ in sensitivity to oxygen, and a range of susceptibility to low O_2 is often found amongst the individuals of a community. Oxygen tolerance tends to be correlated with habitat, so that individuals normally living in well-oxygenated water are less tolerant of low dissolved oxygen than those encountering it periodically. Furthermore, there is some evidence that oxygen-dependent respiration may apply to oxygen uptake relations of communities, as well as to individuals.

Difficulty is encountered in determining safe levels of dissolved oxygen for aquatic invertebrates because of i) the wide range of responses reported, ii) the ability of many organisms to inhabit low oxygen regimes and obtain energy anaerobically, and iii) by a lack of understanding of the physiological effects of low O_2. Survival in continuous levels of low oxygen depends on ability to produce energy continuously anaerobically, or the capacity to extract oxygen from an environment low in oxygen. Also, the ability of organisms to cope with oscillating oxygen levels is very poorly known or understood. Obviously, the tolerance of the species involved and the duration of low oxygen condition are of prime importance to survival. Utilization of anaerobic metabolism could satisfy the energy needs of many aquatic invertebrates for long periods, particularly the smaller, sluggish forms with a typically low oxygen demand. The larger, more active

TABLE 7. Pelagic invertebrate species inhabiting an oxygen minimum layer (approx. 0.4 ml O_2/liter = 0.57 mg/liter) in Saanich Inlet, B.C. (from Hoos 1970).

Phylum	Species	Abundance[a]
Cnidaria	*Aglantha digitals*	seasonally abundant
	Aequorea aequorea	rare
	Phialidium gregarium	common
	Aegina rosea	rare
Ctneophora	*Pleurobrachiia pileus*	seasonally abundant
	Beroe cucumis	rare
Annelida	*Tomopteris renata*	rare
Chaetognatha	*Sagitta elegans*	rare
Mollusca Pteropoda	*Clione limacina*	rare
	Limacina helicina	common
Arthropoda Copepoda	*Calanus plumchrus*	very abundant
	Calanus cristatus	rare
	Pseudocalanus minutus	abundant
	Eucalanus bungii bungii	common
	Euchaeta japonica	abundant
	Metridia lucens	abundant
Mysidacea	*Neomysis rayii*	rare
Amphipoda	*Parathemisto pacifica*	abundant
	Euprimno abyssalis	rare
	Cyphocaris challengeri	abundant
	Orchomenella obtusa	abundant
Euphausiaceae	*Euphausia pacifica*	abundant
	Thysanoessa longipes	common
	Thysanoessa raschii	common
	Thysanoessa spinifer	rare
Decapoda	*Spirontocaris sica*	rare
	Munida quadrispina	common

[a]Terms described by Fulton (1968).

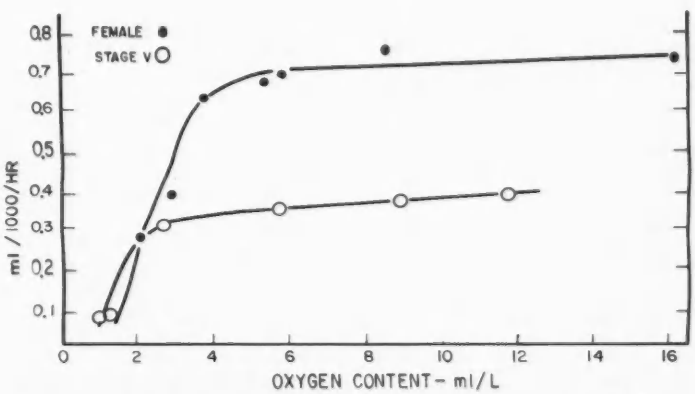

FIG. 15. Effect of oxygen content on the oxygen consumption of female and stage V *Calanus*, at 15 C (from Marshall et al. 1935).

forms are likely to be more affected by low O_2 and thus may be the most sensitive members of the community. Thus, a change from an adequate oxygen regime to one of relative O_2 scarcity may result in loss of the more sensitive organisms and a restructuring of the community. New species, tolerant to low oxygen, could move into the community; or those already present in the community could become more numerous to replace the intolerant species. Alternatively, no replacement could take place, leading to a net reduction in biomass and/or alteration of species composition of the community.

In conclusion, and on a common-sense basis alone, it seems likely that any depression of natural oxygen conditions can result in a change in an aquatic invertebrate community. The severity of this change will be related to the species composition, the species sensitivity of low oxygen, the degree of oxygen depression, and other factors acting on the community, such as temperature. Whether a change in an invertebrate community is necessarily bad from an ecological point of view may be very difficult to determine. It may be that a change from one type of community to another may not be necessarily deleterious. On the other hand, if major food chain constituents drop out, the change could be considered deleterious.

Insufficient evidence exists presently to formulate definite dissolved oxygen criteria concerning aquatic invertebrate communities. However, a reasonable basis will probably be assured by following the recommendations for fish populations given here, particularly as many invertebrates are able to temporarily withstand periods of low oxygen. It must be remembered, however, that any change in dissolved oxygen will likely affect invertebrate communities in some way. Ideally, we need a good knowledge of community structure in areas threatened by pollution so that important sensitive species are identified and protected. In addition, knowledge of the seasonal oxygen minima for specific areas is required to identify periods when further oxygen depression poses a hazard to aquatic life.

Development of Oxygen Criteria for Fish Populations

The incipient oxygen response levels for fish populations were presented in Tables 4A–D. These tables yield:

1. Oxygen response thresholds for freshwater fishes including nonanadromous salmonids (as the steelhead is a variant of the rainbow trout, it was considered a nonanadromous species for the purpose of this paper).

2. Oxygen response thresholds for marine fishes, including both anadromous and nonanadromous species. All the species of salmon (except rainbow trout and steelhead) are included in this section although they spend some portion of their life in fresh water.

3. Thresholds of oxygen response for salmonid eggs and larvae at various stages of development.

These groupings can be further broken down by including or excluding the salmonids, to thereby derive criteria for nonsalmonid freshwater or marine species.

Statistical Treatment of Data for Deviation of Criteria

The data of Table 4A–D were analyzed by averaging the incipient threshold levels of PO_2, milliliters O_2 per liter, milligrams O_2 per liter, and percent saturation for various fish groups within the tables. The data-groups analyzed appear in Table 8. For each group, the mean average incipient threshold oxygen level was calculated and reported with its standard deviation, standard error, and number of observations. Averaging the data in this way assumes that results will be unaffected by different experimental conditions, fish tested, and factors such as temperature. Variations due to differing experimental conditions should be minimized by the large sample size in most instances.

The effect of temperature on the incipient oxygen threshold was examined to test the validity of the averaging procedure. For the largest body of data (Table 4A), all results showing the incipient oxygen level in milligrams O_2 per liter (Fig. 16) and percent saturation (Fig. 17) were plotted in relation to temperature. From these figures it can be seen that the oxygen content threshold, in milligrams O_2 per liter, is relatively unaffected by temperature; although, higher percentage saturation levels are required at higher temperatures. This is logical since the oxygen content drops as temperature increases. Thus, progressively higher percentage saturation is required at high temperatures to fulfil the oxygen requirements of fish. For the analysis then, oxygen content data (milligrams per liter) rather than percentage saturation data were used in the derivation of criteria.

From the data in Table 8, major fish groups (only those with a large number of observations) were selected (Table 9) for criteria purposes. These groups encompass both salmonid and nonsalmonid species in fresh and salt water, as well as larvae and mature eggs of salmon. Owing to the fact that oxygen levels often fluctuate in

TABLE 8. Mean thresholds of incipient oxygen response thresholds listed in Table 4A–D. Values are means ($\bar{x}$) shown with ±1 standard deviation (SD), ± standard error (SE), and the number of observations (n).

Averaged Group		PO_2-mm Hg	Mg O_2/liter	% Satn.
Freshwater species	$\bar{x}$	85.56	5.26	54.62
including salmonids	SD	27.37	2.01	17.53
	SE	5.37	0.37	3.44
	n	26	30	26
Freshwater species	$\bar{x}$	72.56	3.98	46.53
excluding salmonids	SD	18.83	1.65	12.34
	SE	7.12	0.50	4.66
	n	7	11	7
Freshwater salmonids	$\bar{x}$	90.35	6.00	57.60
	SD	28.84	1.84	18.47
	SE	6.62	0.42	4.24
	n	19	19	19
Marine, nonanadromous	$\bar{x}$	111.03	6.72	73.25
	SD	31.54	2.12	19.72
	SE	11.15	0.80	7.45
	n	8	7	7
Marine anadromous	$\bar{x}$	122.86	6.43	78.49
species, including	SD	35.86	2.57	23.03
salmonids	SE	11.95	0.69	7.68
	n	9	14	9
Marine anadromous	$\bar{x}$	—	3.38	—
species, excluding	SD	—	—	—
salmonids	SE	—	—	—
	n	—	2	—
Marine anadromous	$\bar{x}$	122.86	6.94	78.49
salmonids	SD	35.86	2.39	23.03
	SE	11.95	0.69	7.67
	n	9	12	9
Early eggs, freshwater	$\bar{x}$	15.24	1.14	9.65
salmonids	SD	6.40	0.47	4.06
	SE	3.69	0.27	2.34
	n	3	3	3
Mature eggs,	$\bar{x}$	76.47	5.93	48.40
prehatching salmonids	SD	19.00	1.01	12.15
	SE	10.97	0.58	7.01
	n	3	3	3
Hatching eggs and	$\bar{x}$	119.88	8.09	76.26
larval salmonids	SD	35.45	1.65	22.88
	SE	20.46	0.95	13.21
	n	3	3	3
Eggs, fertilization	$\bar{x}$	77.45	3.86	48.95
to hatching, non-	SD	—	—	—
salmonids	SE	—	—	—
	n	2	2	2

natural waters, the levels of protection selected were defined as MINIMUM oxygen levels in a body of water SEASONALLY (fall, winter, spring, and summer). Three levels of protection were devised to provide flexibility in interpretation:

Level A — This level is 1 SD above the mean average incipient oxygen response level for the group. The rationale is that few members of a fish population, or fish community, will likely exhibit effects of low oxygen at or above this level. Level A represents more or less ideal conditions and permits little depression of oxygen from full saturation. It represents a level that

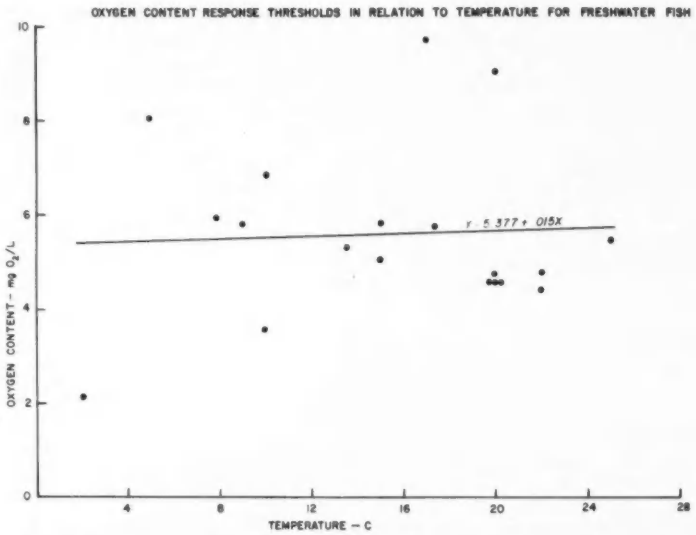

FIG. 16. Incipient sublethal oxygen response thresholds vs. temperature for all the freshwater fish data in Table 4, plotted in terms of oxygen content. The plot is similar to Fig. 17 and shows that the relationship between response threshold is not as clearly related to temperature as when plotted in terms of percentage saturation.

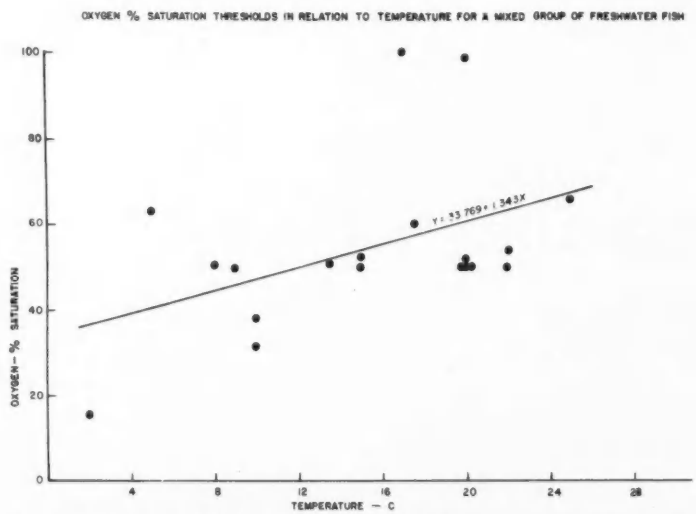

FIG. 17. Incipient sublethal response thresholds vs. temperature for all the freshwater fish data from Table 4, plotted as percentage oxygen saturation. The linear regression line and equation are given. This graph illustrates the effect of temperature on the response threshold expressed in percentage saturation O_2. (Note — As regression of percentile data is a questionable statistical procedure, the regression analysis was repeated following arcsine transformation according to Snedecor (1965). This analysis reduced the slope of the regression line somewhat; however, more slope was present than for the data of Fig. 16.)

TABLE 9. Incipient dissolved oxygen response thresholds selected from Table 8 for derivation of oxygen criteria. These selections were made to give a good representation of fish groups in both fresh and salt water for criteria purposes. Levels of protection A, B, or C (see text) are given. The average temperature, extracted from Tables 4A–D, which corresponds with the average incipient oxygen threshold (in mm Hg PO_2) is indicated in parentheses.

Group	Protection level	PO_2	mg O_2/liter
Mixed freshwater fish population including salmonids	A	113	7.27
	B	86 (15 C)	5.26
	C	58	3.25
Mixed freshwater fish population with no salmonids	A	92	5.63
	B	73 (18 C)	3.98
	C	54	2.33
Freshwater salmonids (including steelhead)	A	119	7.84
	B	90 (15 C)	6.00
	C	61	4.16
Salmonid larvae and mature eggs	A	155	9.74
	B	120 (9 C)	8.09
	C	85	6.44
Marine, nonanadromous species	A	143	8.84
	B	111 (11 C)	6.72
	C	80	4.60
Marine, anadromous species, including salmonids	A	159	9.00
	B	123 (17 C)	6.43
	C	87	3.86
Anadromous salmonids in sea water	A	159	9.33
	B	123 (17 C)	6.94
	C	87	4.55

assures a high degree of safety for very important fish stocks in prime areas.

Level B — This level represents the oxygen value where the average member of a species in a fish community starts to exhibit symptoms of oxygen distress. These values are derived from the class mean averages from Table 8. Some degree of risk to a portion of fish populations exists at this level if the oxygen minimum period is prolonged beyond a few hours.

Level C — At this level a large portion of a given fish population or fish community may be affected by low oxygen. This deleterious effect may be severe, especially if the oxygen minimum is prolonged beyond a very few hours. The values are 1 SD below the B level, or class average, for the group. This level should be applied only if fish populations in an area are judged hardy or of marginal significance, or of marginal economic importance and, as such, are dispensible (i.e. in the socioeconomic sense).

The use of standard deviations to recommend levels of protection is based on the statistical concept that, in normally distributed data, approximately 68% of the values lie with ± 1 SD of the mean. Thus, the recommended levels span the range of responses that include both sensitive and insensitive animals, both within and between species.[1] Analysis of the data for the freshwater fish group, including salmonids, indicates that these data are normally distributed about the mean and yields a typical cumulative frequency curve about the mean (Fig. 18).

The criteria values of Table 9 based on oxygen content in milligrams O_2 per liter and PO_2 were rounded off and entered in Table 10. This table constitutes the final derivation of the recommended criteria. Oxygen content values in milliliters O_2 per liter were calculated from the values for milligrams O_2 per liter in Table 10, and per-

[1]A weaker form of these criteria could be adopted by setting the value of A or B level as the average 24-h O_2 value and the minimum O_2 concentration over any 24 h equal to the next lower criteria level. For example, the value of A would represent the average 24-h O_2 level and at no time should it be allowed to fall below the value of B. Adoption of this scheme could risk serious damage to fish populations at level B. Objections to the use of average values are discussed in Warren et al. (1973).

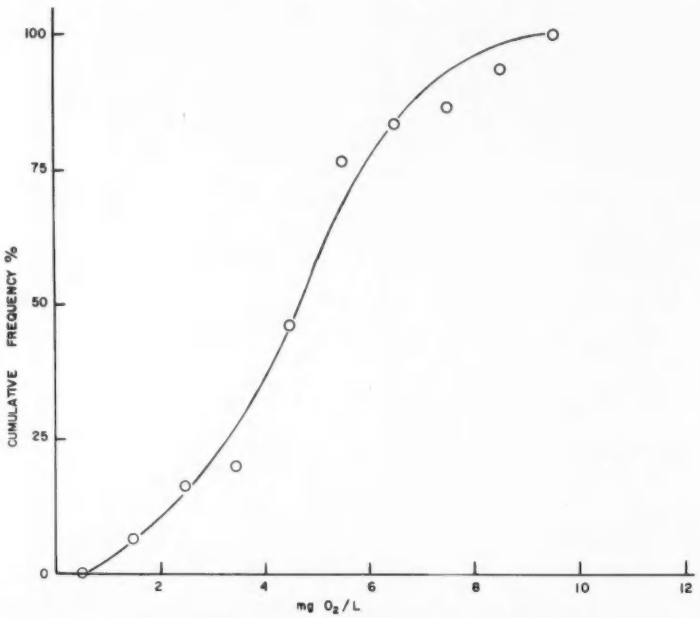

FIG. 18. Frequency distribution of oxygen response thresholds for the largest data group — that for the freshwater fish in Table 4, illustrating that the thresholds follow a more-or-less normal distribution.

cent saturation was calculated for a range of 0–25 C from the desirable PO_2 and content data. Calculations of percent saturation in sea water were based on a salinity of 28‰ (this detailed account of procedures used to derive criteria is given so that others may use the same data to derive their own criteria in another way, if so desired).

It must be emphasized that, as pointed out earlier in this report, fish require both the correct oxygen tension gradient to move O_2 into the blood and sufficient oxygen (per unit volume of water breathed) to fulfill the requirements of metabolism. Therefore, the criteria have been derived to provide both these essential requirements of fish. They are expressed as percentage saturation values over a range of temperatures. At the lower temperatures where water solubility of oxygen is high, the criteria were set using the PO_2 values in Table 10 to ensure that a percentage saturation value consistent with the required PO_2 gradient was present. At the higher temperatures, higher percentage saturation values were necessary to provide the oxygen content requirements dictated by the milligrams O_2 per liter values in Table 10.

A major criticism of these criteria might be that they are heavily biased towards the oxygen requirements of salmonids, which tend to be higher than some other species. This results from much key work being confined to salmonids. With this possible criticism in mind, the criteria recommended in Table 10 for nonsalmonid species in fresh and salt water are lower than for salmonids; however, they are based on fewer data. It must be stressed therefore, that although these criteria are based on best-available knowledge to date, they are tentative and should be subject to revision when new information arises.

A key point in application of the criteria presented above is that daily, seasonal, or periodic low oxygen levels may occur naturally in many water bodies. These natural levels may be near or below recommended criteria levels A, B, or C. In such cases, judgement and caution must be exercised in applying criteria to these naturally oxygen-deficient waters. A standard could be imposed which would be impossible to meet owing to natural fluctuations in dissolved oxygen. Also, if natural depression of oxygen occurs in waters that are to receive wastes with a significant oxygen demand, even a relatively small discharge could reduce oxygen below critical levels. One method of assessing the severity of hazard would

TABLE 10. Oxygen criteria based on percentage saturation values derived with three levels of protection as outlined in the text. PO_2's and values of mg O_2/liter were extracted from Table 9 and rounded off for use here. The values shown for milliliters O_2/liter were calculated from the values of milligrams O_2/liter in this table.

The criteria essential for protection of aquatic fish populations are expressed as percentage saturation values at various temperatures. They were derived from both PO_2 and mg O_2/liter values, as both oxygen tension and oxygen content are critical factors. At the lower temperatures, the percentage saturation value was determined using the PO_2 values essential for maintaining the necessary oxygen tension gradient between water and blood for proper gas exchange. Higher percentage saturation values are necessary at the higher temperatures to provide sufficient oxygen content to meet the requirements of respiration as defined by the mg O_2/liter values.

Percentage saturation values are defined as "oxygen minima" at each level of protection. Graphical presentation of the results is found in Fig. 19. The temperatures corresponding to the percentage saturation criteria are defined as "seasonal temperature maxima."

Group	Protection level	PO_2	ml O_2/liter	mg O_2/liter	% Satn. at C for criteria					
					0	5	10	15	20	25
Freshwater mixed fish population including salmonids	A	110	5.08	7.25	69	70	70	71	79	87
	B	85	3.68	5.25	54	54	54	57	54	63
	C	60	2.28	3.25	38	38	38	38	39	39
Freshwater mixed fish population with no salmonids	A	95	3.85	5.50	60	60	60	60	60	66
	B	75	2.80	4.00	47	47	47	47	47	48
	C	55	1.75	2.50	35	35	35	35	35	36
Freshwater salmonid population (including steelhead)	A	120	5.43	7.75	76	76	76	76	85	93
	B	90	4.20	6.00	57	57	57	59	65	72
	C	60	2.98	4.25	38	38	38	42	46	51
Salmonid larvae and mature eggs of salmonids	A	155	6.83	9.75	98	98	98	98	100	100
	B	120	5.60	8.00	76	76	76	79	87	95
	C	85	4.55	6.50	54	54	57	64	71	78
Marine, nonanadromous species[a]	A	140	6.13	8.75	88	88	95	100	100	100
	B	110	4.73	6.75	69	69	74	82	90	98
	C	80	3.15	4.50	50	51	51	55	60	65
Anadromous marine species, including salmonids[a]	A	160	6.30	9.00	100	100	100	100	100	100
	B	125	4.55	6.50	79	79	79	79	87	94
	C	90	2.80	4.00	57	57	57	57	57	58

[a]Percentage saturation calculations based on salinity of 28‰.

be to calculate the expected oxygen depression for the receiving water and then subtract this depression from the naturally occurring O_2 minimum value(s). The severity of hazard could then be estimated by reference to Table 10, after conversion of the data to percentage saturation (see also Fig. 19).

Guidelines for Low Oxygen Tolerance of Aquatic Invertebrates

Owing to considerable lack of knowledge of effects of low oxygen on the physiology of marine invertebrates, oxygen criteria cannot be proposed for these organisms at this time. It is reasonable to assume, however, that the levels proposed in Table 10 for fish would provide good protection for most aquatic invertebrates. Indeed, many invertebrates can withstand a much more severe oxygen lack for a longer period than can fish. Regarding aquatic invertebrates and aquatic ecosystems generally, the following recommendations are proposed:

1. Any change in the dissolved oxygen regime will likely have some effect on the ecosystem and the magnitude of that effect will depend on the severity and duration of the change.

2. A guide to sensitivity of the system could be based on knowledge and study of the area in question. Open ocean coasts, swift-flowing freshwater streams, or other well-aerated bodies of water are likely to contain more low oxygen-intolerant species than poorly aerated water bodies. Thus the habitat and its physical features would serve as an index of the degree of sensitivity of the system.

3. Wherever possible, knowledge of the natural seasonal oxygen *minima* conditions should be available as a guide to implementing criteria.

4. Where seasonal oxygen minima are identified, it is suggested that the potential depression

of water oxygen caused by a discharge be calculated. In this way, further reduction in oxygen level, imposed on the natural condition, could be predicted.

Relationship of Proposed Criteria to Other Documents

Davis (1975) summarized the findings of other oxygen criteria documents. The reader should consult that reference for a detailed comparison of various points of view. A number of the other documents are based on the comprehensive review of Doudoroff and Shumway (1970).

This report was written entirely without consulting other criteria documents prior to derivation of the criteria, hopefully, to avoid bias. I was generally aware, however, of the findings of other reviewers and it was partly for this reason that the foregoing statistical methods were used to derive cause/effect criteria for the present report. I was also aware that Doudoroff and Shumway (1970) had recommended various "levels of protection" in compiling oxygen criteria and that full acknowledgement must be given to their sensible concepts.

In general, the O_2 criteria formulated in this report, and derived specifically for Canadian aquatic life, incorporate a number of considerations identified as critical in other oxygen criteria documents. The herein-presented criteria include the concept of seasonal oxygen minima, allow consideration of temperature, and contain three levels for assessment of potential risk: Level A provides a high safeguard for very important aquatic populations; with Level B, there is some possibility of moderate risk to aquatic organisms, because it allows some degree of oxygen depression; with Level C, it is possible that a large portion of a given fish population or fish community may be severely affected by low oxygen, especially on a "chronic exposure" basis.

These criteria, unlike most others, present oxygen levels as percentage saturation. This was

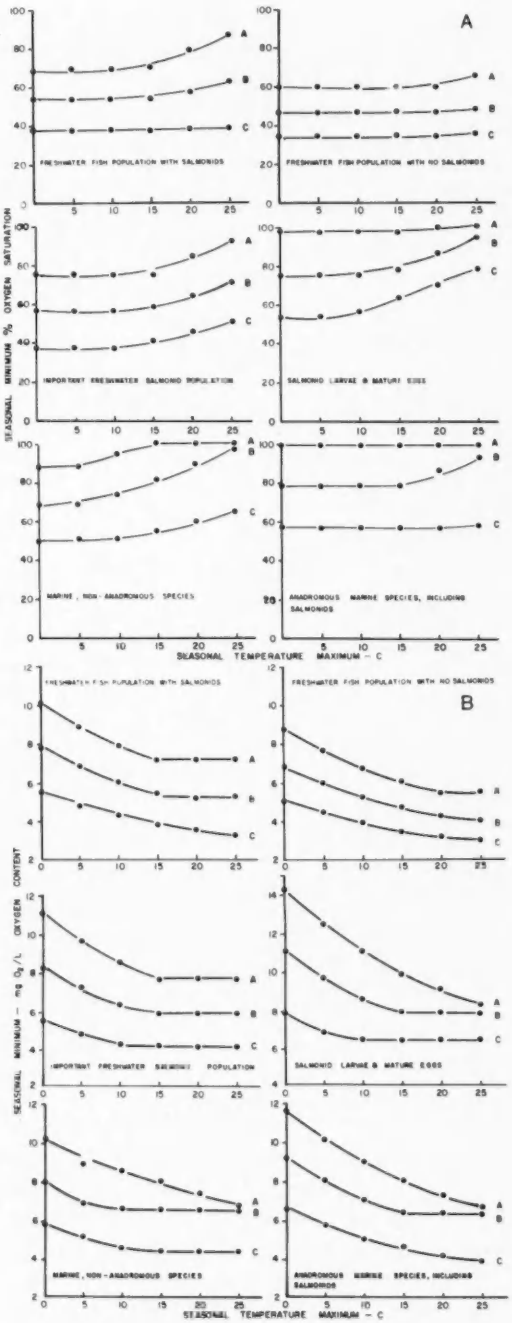
A
B
SEASONAL MINIMUM % OXYGEN SATURATION
SEASONAL MINIMUM – mg O2/L OXYGEN CONTENT
SEASONAL TEMPERATURE MAXIMUM – C
FRESHWATER FISH POPULATION WITH SALMONIDS
FRESHWATER FISH POPULATION WITH NO SALMONIDS
IMPORTANT FRESHWATER SALMONID POPULATION
SALMONID LARVAE & MATURE EGGS
MARINE, NON-ANADROMOUS SPECIES
ANADROMOUS MARINE SPECIES, INCLUDING SALMONIDS

FIG. 19. A, Oxygen criteria, expressed as seasonal minima in percent saturation, at various seasonal temperature maxima. Three levels of protection, A, B, and C, are given as defined in the text. Values used in derivation of the criteria are summarized in Table 10; B, Oxygen criteria, expressed as oxygen minima in mg O_2/liter (ppm), at various seasonal temperature maxima. Three levels of protection, A, B, and C, are given as defined in the text. These values were calculated from the percentage saturation values illustrated in (A) and summarized in Table 10.

done partly because the concept is easily understood and can be assessed conveniently. In addition, the criteria, expressed as percentage saturation, incorporate both the necessary oxygen content and partial pressure essential for fish respiration over a range of temperatures. The values can be easily converted to other units such as milligrams O_2 per liter.

Unlike other criteria, the present oxygen criteria include consideration of marine fish, based on actual data. These data are admittedly somewhat scanty; thus, there is a need for more extensive data on all aspects of pollution research with marine fish.

Lacking current knowledge of oxygen requirements for aquatic invertebrates, oxygen requirements for assessment of harm to these organisms have not been specifically proposed. For protection of aquatic invertebrates, adoption of the criteria relating to fish populations is recommended, for reasons given in this report. Much more work is needed in this particular area, to elucidate the effects of low oxygen on invertebrate communities and ecosystems, especially those in the sea.

Suggestions for Implementation of Criteria

Criteria should only be implemented in keeping with a thorough knowledge of the specific area in question and the aquatic life inhabiting it. In my opinion, use of a single oxygen level, or group of oxygen levels, applied to all waters on a national basis is an unrealistic and potentially questionable practice. A sensible approach would be to form regional panels, whose members have expertise in diverse fields such as fisheries biology, ecology, oceanography, limnology, and industrial pollution. The duty of the panels would be to review existing information, initiate studies to seek new knowledge and implement criteria (such as those suggested here) once sufficient knowledge on the relevant area was amassed. The panel should try and establish:

1) What fish and invertebrates species reside in or move through the area, their relative O_2 sensitivity, and what their economic, recreational, or biological importance is.

2) The seasonal natural oxygen regime for the area.

3) Physical factors that affect dissolved oxygen levels such as temperature regimes, water circulation patterns, and "turnover" of water masses.

4) Existing oxygen-depleting factors, either natural or man-made such as industrial or domestic waste discharge, respiration, upwelling of deep O_2-deficient water, decomposition, etc.

5) Occurrence of toxicants in the water mass which, in combination with low oxygen, may lead to a potentiation stress responses on the part of aquatic organisms.

If such a panel implements oxygen criteria in keeping with the above considerations, there is considerable assurance that the criteria will be realistically applied to the area in question according to the best current available knowledge. The proposed criteria should be considered subject to revision as new knowledge comes available.

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Glossary of Terms

Acclimation — compensatory change or adjustment of a bodily function (e.g. metabolism) in response to a single environmental factor, such as temperature, in the laboratory (Prosser and Brown 1962).

Aerobic — process that proceeds in the presence of oxygen such as aerobic respiration (respiration requiring and utilizing oxygen).

Anaerobic — process that proceeds in the absence of oxygen, such as anaerobic respiration (energy production from metabolites without oxygen utilization).

Anadromous — term applied to an animal, usually a fish, that spends part of its life in fresh water and part in salt water, necessitating some movement (migration) from one media to the other.

Blood oxygen dissociation curve — experimentally derived curve describing the saturation kinetics of blood in relation to available oxygen tension. The curve indicates percentage saturation of the blood at different oxygen tensions, illustrating the oxygen level required to saturate the blood and how the blood carries oxygen and delivers it to the tissues.

Criteria — set of rules or guidelines based strictly upon a scientific assessment of environmental harm. Oxygen criteria are derived from a study of cause and effect interrelations involving the responses of organisms to reduced oxygen.

Food conversion efficiency — efficiency with which a given quantity of food consumed is converted to body mass.

Hypoxia — a condition when oxygen availability is low. A fish reacting to low oxygen can be said to be hypoxic.

Incipient — just beginning. The incipient oxygen response threshold can be defined as the oxygen level where some organism just starts to respond in some way to reduced oxygen availability.

Metabolism — chemical process involving enzyme reactions in the body whereby energy is made available for bodily work. Metabolic reactions may be both oxidative and nonoxidative in nature.

Metabolic rate — rate at which metabolism proceeds, usually expressed as oxygen consumption rate, caloric heat produced or rate of CO_2 liberation. In many instances, fish and aquatic invertebrate metabolic rate is measured in terms of oxygen uptake rate.

Oxygen concentration (content) — the concentration, measured in terms of milligrams O_2 present in a given body of water, usually 1 liter, at a specified temperature, pressure, and salinity. The amount of oxygen present per unit water breathed by an aquatic animal governs, in part, the oxygen availability to the organism.

Oxygen partial pressure (PO_2) —oxygen tension measured in millimeters Hg, in a solution. Oxygen diffuses down a concentration gradient across the respiratory surface. The magnitude of the gradient is conveniently determined by measuring oxygen partial pressure on either side of the exchange surface and considering solubility coefficients of oxygen in water and blood.

Phototaxis — directed behavioral response of an organism to light. Phototaxis may be positive (directed towards) or negative (directed away) to the light stimulus.

Respiration — process of gaseous exchange across the respiratory surface via diffusion. Often respiration rate (gas exchange rate) is confused with ventilation rate (rate of breathing movements).

Respiratory dependence — condition exhibited by many animals where, under certain conditions of oxygen availability, respiration rate (and thus aerobic metabolic rate) show a direct correspondence to the available oxygen level. Animals whose oxygen uptake rate increases as oxygen availability increases are thus termed conformers.

Respiratory independence — many animals maintain their oxygen uptake rate independent of available oxygen over a wide range of environmental oxygen concentrations. These animals are said to exhibit respiratory independence and may, in some instances, regulate their oxygen uptake rate. In most cases, however, when available oxygen declines to a certain critical level, oxygen uptake rate declines dramatically, indicating the presence of a limiting situation. Animals that maintain their metabolic rate independent of available oxygen are termed regulators.

Salinity — total salt content of water expressed in parts per thousand (‰).

Standard deviation — statistical term that measures the spread, or deviation, of individual measurements from the center (usually the mean average) of normally distributed data.

Standard error — statistical term describing the standard deviation of sample means for samples drawn from a parent population — i.e. a measure of the reliability of the mean average.

Ventilation rate — breathing rate of an organism denoting the number of ventilatory movements per unit time.

List of Symbols

D.O. — abbreviation for dissolved oxygen level.

H_2O vap. press. — water vapor pressure, in millimeters Hg.

mg O_2/liter — concentration of oxygen in milligrams per liter of water (1 mg O_2/liter = 1 ppm).

ml O_2/liter — concentration of oxygen in milliliters per liter of water (0.7 ml O_2/liter = 1.0 mg O_2/liter = 1 ppm).

n — the number of individual data used in a computation

PCO_2 — partial pressure of carbon dioxide in millimeters Hg.

PO_2 — partial pressure of oxygen in millimeters Hg.

ppm — abbreviation for parts per million concentration term.

SD — standard deviation (statistical terminology).

SE — standard error of the mean (statistical terminology).

MTL — median tolerance limit in terms of dosage or concentration of a substance that is lethal to half of the organisms in a bioassay test over a specified test time — usually 96 h.

‰ — parts per thousand (used for expressing salinity of water).

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Variability in Atlantic Cod (*Gadus morhua*) from the Northeast Atlantic: a Review of Seasonal and Environmental Influences on Various Attributes of the Flesh

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LOVE, R. M. 1975. Variability in Atlantic cod (*Gadus morhua*) from the northeast Atlantic: a review of seasonal and environmental influences on various attributes of the flesh. J. Fish. Res. Board Can. 32: 2333–2342.

Fish to be used as food are judged on the nature of the texture, flavor, odor, surface appearance, and color of the fillet. Investigations on Atlantic cod (*Gadus morhua*) have shown that all of these attributes can vary according to the season and the place where the fish were caught.

Factors that influence one or more of the attributes are: firstly, the postmortem pH of the muscle, itself influenced by the supply of food and in some way by the body length; secondly, the lipid content, also influenced by feeding; and thirdly, the swimming activity of the fish.

LOVE, R. M. 1975. Variability in Atlantic cod (*Gadus morhua*) from the northeast Atlantic: a review of seasonal and environmental influences on various attributes of the flesh. J. Fish. Res. Board Can. 32: 2333–2342.

Les poissons utilisés comme nourriture sont jugés par la structure, la saveur, l'odeur, l'apparence et la couleur de leurs filets. Des recherches sur la morue atlantique (*Gadus morhua*) démontrent que toutes ces qualités peuvent varier suivant la saison et le lieu où le poisson a été capturé.

Les facteurs qui influencent une ou plusieurs de ces qualités sont: premièrement, le pH du muscle post-mortem, lui-même influencé par la quantité de nourriture ingérée et, d'une façon quelconque, par la longueur du corps; deuxièmement, le contenu lipidique du muscle, également influencé par la nourriture; et troisièmement, l'activité natatoire du poisson.

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THE characteristics of fish intended for processing often vary from batch to batch, so that processors can never be sure in advance that a particular consignment can be processed satisfactorily in a particular way. Sometimes all of the fish from a voyage fall to pieces during filleting and have to be made into a less valuable product. Others may be abnormally colored. Some spoil unusually quickly in melting ice, while again others may have a very short life in the cold store before becoming unacceptably tough or off-flavored.

"Seasonal variation," "ground-to-ground variation," "feediness," and other phrases are used to account for sudden changes in properties, but there is almost no systematic information available, and the "explanations" offered are no more satisfactory than the classical one given for fish spoiling more readily than beef — that "they are more perishable."

Observation by factory foremen yields some curious information; for example, plaice (*Pleuronectes platessa*) that are caught along with haddock (*Melanogrammus aeglefinus*) have softer flesh than those caught alone — but again no explanation has been essayed.

What are we to make of the gaping (muscular disintegration) of cod, which on one occasion was seen to decrease with increasing body length when the fish were caught off the Faroe Islands (Fig. 1a) but exhibited no such relationship when they were caught of Iceland in the same week (Fig. 1b), or of the bacterial spoilage in melting ice, which is slower in Faroe Bank cod than in those from the North Cape Bank of Norway (Reay 1957)?

This paper reviews the progress made so far towards ridding the subject of "ground-to-ground variation in quality" of its mystique and discovering how many of the possible factors involved really are important. It may never be practicable to change an unsuitable fish into a suitable one once it has been caught, but it might be possible, by recognizing certain signs, to indicate the products into which a particular batch can most suitably be made.

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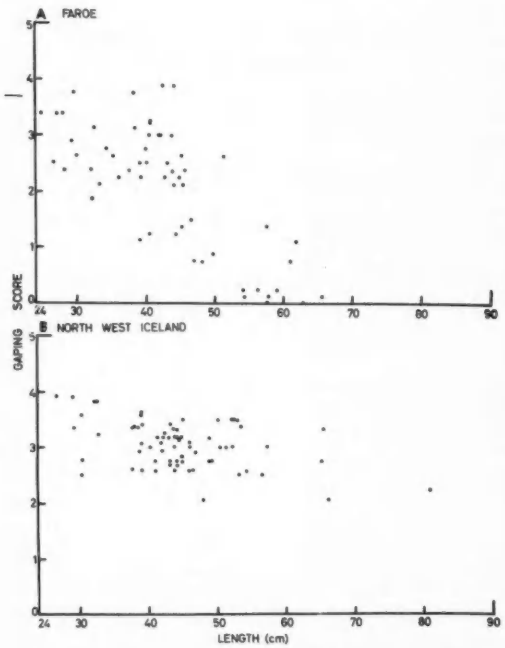

FIG. 1. Gaping, assessed on an arbitrary scale (0 = smooth fillet; 5 = extensive breakup through the thickness of the fillet), in cod of different sizes caught off a) the Faroe Islands and b) NW Iceland at the same time of the year. All fish were gutted, frozen, thawed, and filleted (Love unpublished data).

Characteristics and the Factors that Influence Them

(a) TEXTURE

The texture of cooked cod muscle as eaten is known to be strongly influenced by the pH (Kelly et al. 1966; Cowie and Little 1966). For this reason, Connell and Howgate (1968) mathematically adjusted all their taste panel assessments to a fixed pH before evaluating the toughness caused by cold storage. If the pH is low, the texture is rather tough, and since cold storage causes further toughening, fish of low pH soon become unacceptable if kept frozen. It is, therefore, considered desirable for cod to have a pH of not less than 6.6 (Kelly 1969a) or 6.7 (Kelly 1969b) if they are to be preserved in this way. Indeed, since a fish with a very high pH (over 7.0) has an objectionably sloppy texture, it can actually be improved by cold storage.

The pH discussed in this paper is the minimum pH reached after death, resulting from the enzymic conversion of carbohydrates in the muscle to lactate. Some well-fed cod reach a low pH, while starved or spawned cod remain close to neutrality. The total range in this species after about a day packed in melting ice (6.1 to 7.2) is smaller than that of the pH of post mortem beef muscle (5.4 to 7.2) but is enough to cause marked changes in the texture and other properties.

During the depletion associated with spawning, the water content of cod muscle rises (Love 1960), and in artificially starved fish the rise from normal values (80.0–80.9%) begins, after a delay period, at the same time as the rise in pH (Love 1969). However, the two parameters do not always move in parallel, and by measuring the texture of cod having a wide range of water content but constant pH, Love et al. (1974b) showed that the influence of water content alone was slight (Fig. 2); pH exerted the major influence on texture even where the water content did not change (Fig. 3). The protein content of the muscle, as directly determined, also exerted little influence on the texture. This is perhaps unexpected, in spite of the findings on water which largely mirrors the protein content, since the protein is the very "substance" of the edible flesh. The physical state of the protein is presumably more important than the actual amount present.

Differences in texture were found between batches of fish caught on different grounds, even where the pH was the same. In the case of St. Kilda (W. Scotland) and Fyllas Bank (Greenland) cod, the discrepancy was seen over a whole range of pH values (Fig. 4), so clearly another factor was involved. The St. Kilda fish in this experiment were on the whole larger than those from Fyllas Bank, so as an extension to this work the texture values from a larger number of fish were plotted against the body lengths, taking only fish with muscle water content within the range

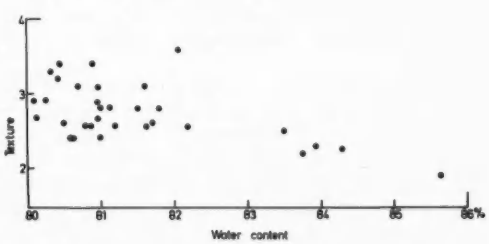

FIG. 2. Texture of cod muscle after cooking, as perceived by a taste panel, in relation to the water content which varies according to the nutritional state of the fish. The pH was in the range 7.0–7.4. A score of 3 is "normal cod," values higher being tougher and values lower being soft or sloppy (Love et al. 1974b).

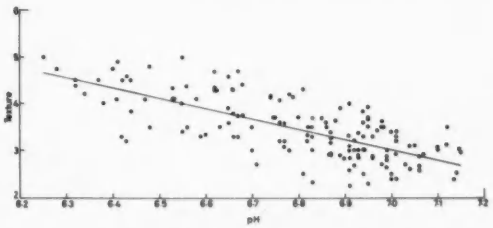

FIG. 3. Texture of cooked cod muscle in relation to the pH, the water content being within the narrow range 80.0–80.9%. Other details as in legend to Fig. 2 (Love et al. 1974b).

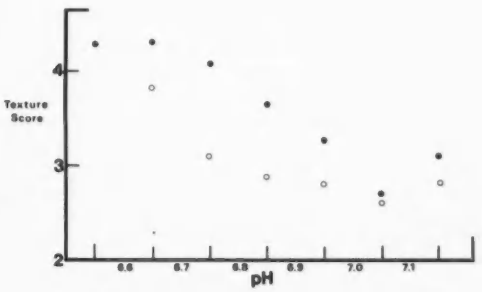

FIG. 4. Texture of cooked cod muscle in relation to pH, in spring-caught fish from two grounds (modified from Love 1969). The upright line on the abscissa between pH values 6.6 and 6.7 points to average texture scores of all fish in the experiment with pH values between 6.60 and 6.69. The next line points to the average of fish with pH between 6.70 and 6.79 and so on. ●, St. Kilda (W. Scotland); ○, Fyllas Bank (W. Greenland).

80.0–80.9% (i.e. nonstarving). In addition, the texture values were mathematically adjusted to a constant pH value of 6.7, so that the effects of size alone could be ascertained.

The results (Fig. 5) show by a *t*-test on the regression coefficient that size does in fact significantly influence the texture, though not to the same extent as pH.

(b) Surface Appearance (Gaping)

Fillets of fish are said to gape when slits or holes appear in the cut surface. Gaping used to be of little consequence to the industry when gutted fish were packed in melting ice on the catching vessel and then filleted and processed ashore, but it has increased in importance with the practice of freezing fish whole at sea and then thawing and filleting later. Under these circumstances the fillets sometimes disintegrate so much that they cannot be mechanically skinned, and are unacceptable to buyers because of their "lace-curtain" appearance. Different species of fish differ in their susceptibility to gaping (Love et al. 1969), but cod itself is subject to wide and hitherto unexplained variations.

We are not concerned here with gaping that results from bad handling, such as straightening fish that were fixed in a bent position in rigor mortis, allowing them to lie in the sun on the deck or freezing them extremely slowly, but as before with factors related to the environment or the life cycle. It is, however, worth pointing out that the longer the fish are kept after death before being frozen the more they gape (Love et al. 1969; Love and Lavéty 1972). I shall return again to this point.

The experience of processors seemed to show that gaping was worst in soft, watery fish that had recently spawned, but systematic study revealed that the most watery fish did not in fact gape at all after freezing and thawing but gaped proportionately more as the fish were better nourished (Love and Robertson 1968). Initially it was believed that, since these fish had been frozen when the pull of the rigor mortis contraction was at a high level (1 day after death), the differences in gaping resulted from differences in the strength of the contraction (starving, weak; nourished, strong). In fact there are other possible explanations.

One is that cod in progressively better nutritional condition have a lower ultimate pH in the muscle (MacCallum et al. 1967). Studies on the breaking strengths of clean strips of myocommata isolated from cod (Love et al. 1972b) have shown that this tissue is extremely sensitive to small changes in pH — a change of even 0.1 pH unit has a measurable effect. The relationship is reversible, weak connective tissue at low pH becoming stronger if the pH of the immersing fluid is raised. It is, however, unlikely that the tendency

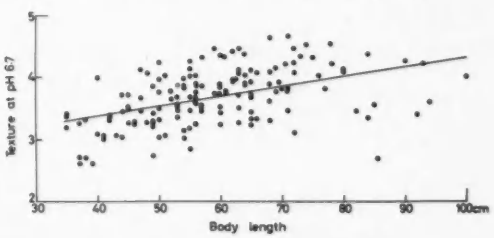

FIG. 5. Regression of texture of cooked cod muscle on body length, the water content being within the range 80.0–80.9% and the pH 6.7. Coefficient of correlation = 0.47. (Love et al. 1974b).

of certain fish to gape could be reversed as an industrial process by, for example, soaking them in weak alkali, if only because of the long time required to change the pH throughout the thickness of the skin and musculature.

Again, Lavéty and Love (1972) have shown that during starvation the myocommata and skin of cod become very much stronger, even when the strength is measured at constant pH. The mechanism of this phenomenon remains to be elucidated, but the tissue thickens (Love and Lavéty unpublished data), perhaps being derived from the breakdown of other tissues. It seems to be an instance of effective adaptation to starvation, as the stronger binding materials would obviously do much to hold the living body together and prevent the disintegration of the soft watery musculature under physical stress. The return to a weaker state when normal feeding is resumed would obviously repay study, since the fish might well gape during the destruction of surplus connective tissue.

Like texture, gaping is also influenced by the size of the fish. Love and Steel (unpublished data, quoted in Love et al. 1972a) showed that in cod there was a linear decrease in gaping with increase in size, but subsequent experiments failed to confirm the results, gaping values in fish of different sizes appearing in a completely haphazard fashion or even with the former relationship shown in reverse. Many experiments later, it finally became clear that the pH effect and the size effect in fact work in opposition to one another, so that the phase of the seasonal cycle in pH (to be discussed later) determines which effect predominates. When gaping values were adjusted to a constant pH, there was always found to be less gaping in bigger fish (Love et al. 1972a).

It is known that the myocommata, and so probably the pericellular connective tissue which cements the myocommata to the myotomes, are thicker in bigger fish (Fig. 6 of Love 1970) so an increase in size appears to confer a structural advantage.

The interplay of the two factors is shown in Fig. 6; whenever there are large and small fish of the same pH, the large fish always gape less, but the pH of the large fish can extend to much lower values so that some of them are made to gape more than small fish from the same batch.

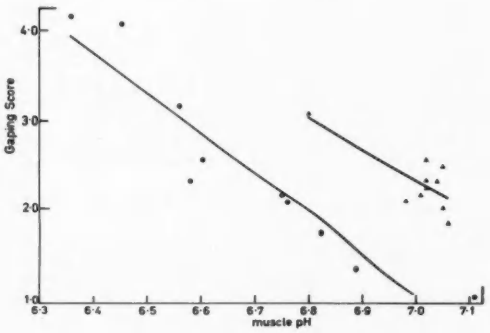

FIG. 6. Gaping in fillets of two size-groups of cod according to pH. (Love et al. 1972a). ▲, Small cod (less than 50 cm); ●, large cod (over 70 cm.)

(c) Flavor and Odor

Frozen fatty fish such as herring or mackerel become unacceptable as food after a period in the cold store because they develop a rancid flavor and odor. This effect leads to their rejection as food before the toughening of the texture has become serious. Cod also gradually develop an unpleasant taste on frozen storage, usually described as a "fish meal," "salt fish," or "wet cardboard" taste. A smell of the same general description can also be perceived in the raw and the cooked material. Recent work has shown that the compound largely responsible is hept-*cis*-4-enal, which can be detected by taste in very low concentrations (McGill 1974; McGill et al. 1974). All indications are that the compound appears as the result of the oxidation of lipids, even in non-fatty fish such as cod where they usually form less than 1% of the flesh.

Other flavors, not associated with cold storage, are found in fish from certain grounds. The "blackberry" flavor described by Sipos and Ackman (1964) results from cod eating large quantities of a marine invertebrate. An undesirable seaweedy taint has been ascribed to cod from Bear Island, and the smell associated with it is said to pervade the factory when such fish are landed. A taste like iodine has also been noted from time to time in cod from a wide range of northern grounds. It seems likely that both these flavors reflect something in the diet, but at present it is not possible to recommend suitable avoiding action.

(d) Spoilage Rate

Explanations for ground-to-ground differences in spoilage rate, e.g. that North Cape Bank cod spoil more rapidly in melting ice than Faroe Bank cod (Reay 1957), are at best conjectural, but there is one pointer. On the two occasions in which 50 fish from both these banks were sampled by the writer's group at the same time of the year, the mean postmortem value of pH was lower in Faroe Bank fish (about 6.6) than in

North Cape Bank fish (about 6.8). These are isolated observations, but in addition it is well known among fishermen that large cod keep better than small, and the former also tend to have lower pH values (e.g. Fig. 6). Spoilage bacteria tend to propagate better at higher pH values (Jay 1970), so here perhaps is a partial explanation. There are other possibilities, however, such as the higher loading of the muscle of the arctic fish with trimethylamine oxide (Shewan 1951), leading presumably to a stronger trimethylamine flavor during a similar period of spoilage.

(e) Color

The flesh of cod immediately under the skin is of a reddish-brown color, much of which is due to the presence of haem pigments within the muscle, so that bleeding the fish before processing does not change the situation. Processors have reported considerable variations in the intensity of color which sometimes, for aesthetic reasons, renders large quantities of fish unsuitable for certain products. "Seasonal variation" is again invoked as explanation.

Love et al. (1974a) measured the color of the total extracted haem pigments in the dark muscle and found that the intensity of the brown pigmentation varied according to the locality of catching. Cod from two grounds off Spitzbergen were the most deeply colored, followed by those from Bear Island, those from six other grounds were less so, differing little among themselves, and those from the Faroe Bank were palest. Now cod from the Barents Sea are unusual in that they migrate great distances each year (Trout 1957), swimming southward down the Norwegian coast where they spawn in February. In this first experiment they had been caught in September after their return. In a subsequent trip made in July 1973 the color of the lateral muscle of Spitzbergen cod was similar to that found previously (Love et al. 1974a), but those just north of the northern tip of Norway were darker still. It is tempting to conclude that, 2 mo earlier, these fish were the migratory stock that had still not reached home. A further voyage has added support to the general finding: the dark muscle of Spitzbergen and Bear Island cod was darkest of those caught in September 1974, while Faroe Bank cod were palest. Perhaps related to the pale color is the fact that Faroe Bank cod are a known stationary stock (Jones 1966; Jamieson and Jones 1967). Cod from the Minch (W. Scotland), another stationary stock, have been found (September 1972) to be paler still.

In spite of a certain conflict of views it is generally accepted that the dark strip of muscle along the side of fish is used for cruising, i.e. habitual swimming, while the main mass of white muscle is kept in reserve, like a coiled spring, for the sudden dash in pursuit or escape (Boddeke et al. 1959; Bone 1966; Love 1970). It is not unreasonable to suppose that extra activity in migration would result in the development of extra oxygen-carrying capacity in the dark muscle by means of a greater concentration of haem pigments.

We have now seen that the principal attributes of cod are influenced by the pH after death, the food supply as seen in the lipid content and certain "taint" flavors, the body length and the amount of habitual swimming undertaken by the fish.

The second part of this paper reviews evidence of variability in these influences themselves.

Variations in the Causative Factors

(a) pH

It will now be clear that the pH of the muscle is probably the most important single factor controlling the acceptability of the product, since if it is low the fish will gape and will be too tough to cold-store for long, though they may keep better in melting ice.

After the death of the fish, at least some of the residual muscle glycogen is converted into lactate, which causes a fall in pH. While there may be a good correlation between muscle glycogen at death and a subsequent pH drop, the relationship does not always hold (MacCallum et al. 1967), so other factors may be involved. On the other hand, the correlation between lactate concentration and pH holds true under a variety of postmortem conditions whether the fish were frozen or not (MacCallum et al. 1968; Kida and Tamoto 1969), and work on cod from different grounds seems to show that no additional information is obtained from lactate determinations if the pH is known (Love and Robertson unpublished data). The postmortem pH may vary with different amounts of struggling on capture (Leivestad et al. 1957), but the effect is surprisingly difficult to reproduce experimentally (Love and Muslemuddin 1972) and will not be considered further here, except to point out that different fishing methods, e.g. line, trawling, or trapping, probably do produce differences in the pH.

Increases in the concentrations of liver glycogen have been shown to correspond with lower muscle pH values (Love et al. 1974a), so we should probably look to nutritional factors if we want

indications of how the fish will behave on processing.

Cod taken during the winter time almost always show high pH values and raised water contents in the muscle, so presumably they have access to insufficient food. It is probably irrelevant to argue whether they eat fairly intensively throughout the year (Rae 1967) or almost cease feeding in the months before spawning (Stanek 1973) — they still do not absorb enough nutriment to satisfy the needs of maturation. The fish certainly do feed with unusual intensity once the food has become plentiful again in the spring or early summer. The onset of heavy feeding seems to occur quite suddenly, and one can find distended fish almost rectangular in shape. They are undiscriminating at this time, and sand eels (*Ammodytes* sp.), herring larvae, and many other organisms can be found in the stomachs. Only later in the year do they feed mostly on small crustaceans and crabs (large cod eat fish also).

The effect of heavy feeding on the pH of the muscle is dramatic. While virtually all the fish caught may have a high pH immediately beforehand, the whole emphasis can change in just 3 wk, perhaps less, in fish from the same locality (Fig. 7).

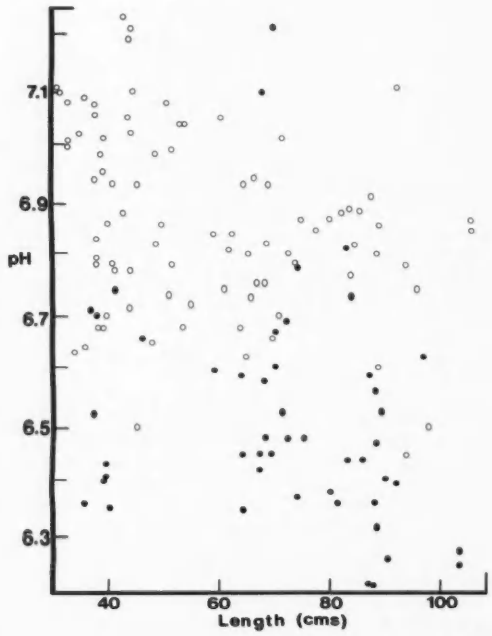

FIG. 7. Postmortem pH of various sizes of cod caught near Aberdeen (Love unpublished data). These fish do not appear to migrate, but the largest size-group can be obtained only at certain times of the year. ○, Caught May 22, 1969; ●, caught June 12 and 15, 1969.

In fish from Tod Head (E. Scotland), a sharp drop came at the beginning of June in 1969, 1971, 1972, and 1973 and in May 1974 and 1975. The pH remained low for about 3 wk–2 mo in different years. The mechanism is not understood and the picture is blurred by the fact that one is dealing at each catching date with different actual specimens. It seems to be a fact, however, that the pH does not remain low for the whole summer, but rises again while food is still plentiful. It is perhaps relevant that when Murat et al. (1972) fed starving carp (*Cyprinus carpio*) on a diet containing glucose, the glucose levels in blood and muscle rose for just 1 or 2 wk and then declined. Perhaps the decline coincided with the restoration of "normal" working of the pancreas.

The period of heavy feeding would be expected to vary from ground to ground, and it is interesting to compare the period of low pH in Tod Head cod (May–June) with that of Newfoundland cod, which was reported by MacCallum et al. (1967) to occur in the third week of July or later.

After spawning, fish are in their worst condition with very soft, watery flesh. The period of unusually heavy feeding in late spring is short, and during that time the flesh has still not had time to recover, so fishermen tend to attribute the wateriness to the heavy feeding — a fallacy, of course. While the large outpouring of digestive enzymes into the gut at this time might reduce the shelf life of fish packed in melting ice without proper washing, we may regard "feediness" as a single-minded attempt by the fish to recover, and not as the cause of soft flesh. It is indeed probable that the traditional association of gaping with the wateriness that follows spawning has come about because of the drop in muscle pH associated with heavy feeding in fish that had not fully recovered.

Both texture and gaping are related to the size of the fish. Any relationship between size and pH seemed likely to be complicated by the greater depletion of energy reserves which is suffered by bigger fish at spawning, because they produce a greater weight of eggs, proportionately to their body weight, as they get older (Orton 1929; Love 1960; May 1967).

The first attempt to relate muscle pH to body length in fish from several grounds (Fig. 8) showed that all values of pH could be found in fish of all sizes. Unfortunately, the fish from any one ground were usually restricted to a narrow size-range, but those from the Faroe Bank covered

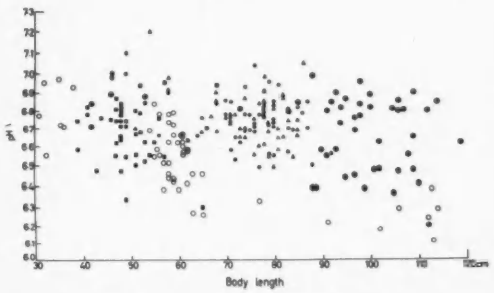

FIG. 8. Postmortem pH of cod of different sizes caught on various grounds in September 1971 (Love et al. 1974b). ○, Faroe Bank; ●, North Cape (Norway); □, Aberdeen Bank; △, Scolpen Bank (White Sea); ⬡, Bear Island.

the whole gamut and seemed to show (hollow circles, Fig. 8) that no large fish had a high pH. A further trip the following year, this time to Faroe Bank alone, confirmed the findings (Fig. 9) and showed that the pH fell steadily as the body length increased until a value of about 6.2 had been reached when the curve leveled out. It was thought at first that the enzymes producing lactate from glycogen were probably inactivated below this pH, but subsequent experiments have sometimes shown the "horizontal" zone lying as high as pH 6.6, so another explanation seems to be needed.

It is clear from the numbers of high pH values that many of the fish illustrated in Fig. 8, especially the larger ones, were suffering from depletion. Relationships like that shown in Fig. 9 would be seen only if the fish were adequately nourished — the Faroe Bank cod is noted for a rapid growth rate, and presumably, a plentiful food supply (Jones 1966; Jamieson and Jones 1967). Apparently, therefore, the pH decreases in fish with an increase in body size down to a limiting value of about 6.2, provided that all the specimens are well nourished. The reason for

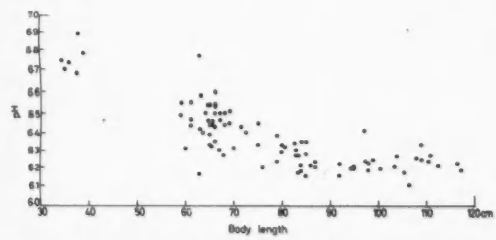

FIG. 9. Postmortem pH of cod caught in September 1972 on the Faroe Bank only (Love et al. 1974b).

the relationship with size is not yet known, but since big fish are annually depleted more severely than small ones, it is not unreasonable to expect them to lay down greater energy reserves to give them some protection (Love 1970).

(b) Lipid

Lipids of cod have generally been ignored in any consideration of suitability as food, since they comprise only about 1% of the wet weight of the flesh. However, well-defined seasonal cycles have in fact been observed in Canadian cod, with minimum values in April (Dambergs 1964; Castell and Bishop 1973). Since cold-storage off-flavors are probably the result of the oxidation of lipid, then the off-flavor may well vary seasonally. Supporting evidence comes from the study of different grounds. Cod from the Faroe Bank had the strongest off-flavors and also off-odors of fish caught on five different grounds in the spring of 1970 and cold-stored for 3 mo at −30 C (Table 1). Subsequently, Love et al. (1975) and Ross and Love (unpublished) have shown that Faroe Bank cod do in fact contain more lipid in the muscle than do cod from Aberdeen Bank, one of the groups shown to develop less off-flavor. The total lipids of the white muscle of Faroe Bank cod were found by Love et al. (1975) to increase from 0.63% in June to 0.78% in September, while those from the Aberdeen Bank were at a lower level on each occasion, 0.55 and 0.67%, respectively. The overall seasonal increase in each case resulted from an absolute increase in the phospholipid fraction, and it seems most likely that hept-*cis*-4-enal is derived from the oxidation of phospholipid rather than neutral lipid (A. S. McGill personal communication). Since there is a seasonal drop in muscle lipid even in Faroe Bank cod, one can envisage a time at which these fish would not develop the cold-store flavor so quickly, and further work is proceeding with this possibility in mind. There is a chance that cold-store flavor will differ between the sexes at certain seasons, since it has been shown in haddock that females mobilize more lipid during maturation than do the males (Shevchenko 1972), but whether or not there is preferential mobilization of certain lipid fractions, leading to the development of less cold-store flavor (or more) in the muscle is unknown. These remarks about cold-store flavor presumably also apply throughout to cold-store odor.

(c) Activity

It is still not certain whether the activity and pigmentation of cod dark muscle vary during the

TABLE 1. Assessment by a panel of the "cold store flavor" and "cold store odor" of cod caught in the spring of 1970 on different grounds, gutted, and stored for 3 mon at −30°. The fish were filleted and steam-cooked before tasting. The scale ranges from 0 = absent to 5 = very strong, the upper limit of commercial acceptability being about 3. Mean values are the scores of five tasters on eight fish. The data are unpublished data by Dr K. J. Whittle of this laboratory. (N = 8 throughout. Comparing the five grounds by analysis of variance by F test showed that the mean of Faroe Bank results were significantly different from the others for both flavor [$P < .01$] and odor [$P < .05$].)

		Cold store flavor		Cold store odor	
Fishing ground	Map reference	$\bar{X}$	SD	$\bar{X}$	SD
Aberdeen Bank	57–05 N 01–15 W	1.68	0.60	1.32	0.51
Faroe Bank	60–53 N 08–20 W	3.02	0.95	2.29	0.84
Faroe Plateau	62–34 N 06–24 W	1.45	0.63	0.91	0.51
SE Iceland	65–27 N 13–08 W	1.70	0.49	1.04	0.35
NW Iceland	65–35 N 25–00 W	1.37	0.37	0.84	0.24

year. Suffice at this stage of the investigation to say that fish intended for processing that are required to have a near-white color can be obtained by avoiding migratory stocks. The only one known to the writer that migrates long distances is the Barents Sea stock, but this covers quite a number of commercial fishing grounds, from the East Barents Sea, Spitzbergen, and Bear Island to the Lofoten Islands at certain times of the year.

(d) SIZE

While one cannot choose the size of cod to be caught in commercial fishing, it is useful to know the effects of size on gaping and texture. We have seen that small fish gape more than large, other factors being equal. Also, that the longer any fish are kept before freezing, the more they gape. It can, therefore, be helpful, if one cannot freeze the entire haul prerigor, to freeze the smaller fish first, since the larger ones can withstand the delay better (Love 1973).

Smaller fish will also tend to retain an acceptable texture for a longer period in the frozen state, because they are intrinsically less tough (Fig. 5) and tend to have a higher pH anyway (Fig. 6). On the other hand, the effects of surface drying in the cold store are more severe in smaller fish because of the higher surface:volume ratio, so they need to be wrapped if a long spell at subzero temperatures is envisaged.

Conclusions

The overwhelming importance of pH in relation to the acceptability of the texture and appearance of the product ensures pride of place for this factor, and so for the nutritional condition of the fish.

The studies over several years on cod from one ground have shown that the period for which the pH is unacceptably low in nearly all of the catch is fairly short, coinciding as it does with the period of heavy feeding at the start of the summer season. It is important to identify this period for the major commercial grounds, because it is likely to vary among them.

The ultimate aim of the work is to enable catchers to be able to decide the most appropriate use for particular batches of fish. Investigations are, however, likely to be protracted, because year-to-year differences may well confuse general conclusions about individual grounds. Indeed, in September 1974 the pH values of all sizes of cod from the Faroe Bank were for the first time found to be "acceptably" high, although the actual date of catching, September 24, was within 1 day of the catching date of the previous year. The reason is unknown except in the usual general terms.

There is a pressing need for methods for assessing the nutritional state of the fish, preferably methods that can be carried out at sea. The biologists' weight:length ratio

$$\frac{(W \times 100)}{L^3}$$

is probably too insensitive and requires the fish to be weighed on a moving boat. Determining the water content of the muscle again requires weighing at sea and is slow, and in any case does not necessarily reflect acceptability.

It is possible to use a digital pH meter on a boat, and here lies some hope. On the other hand, the fish must have been dead for about a day for the pH reading to be meaningful, by which time the catching vessel may have moved to another ground.

How do we identify immediately a fish that has been feeding recently? The fullness of the stomach is often quoted, but may not help if the method of capture causes vomiting, which it frequently does.

A tentative suggestion is that the color of the bile in the gall bladder may give a useful clue under seagoing conditions. The bile in starving cod is green or dark blue (an effect noted in starving freshwater species by Créac'h 1964) and the gall bladder becomes progressively bigger and darker as starvation progresses. In actively feeding fish, the bile exhibits a pale straw color, but observations in 1974 have shown that it begins to turn green if the fish are kept alive without food for as little as 9 h after capture. We may, therefore distinguish between fish that were eating up to 9 h before being caught and those starving for a longer period.

The size and color of the liver gives a good indication of the oil content — small reddish livers characterize starving fish while large yellow livers are full of oil. However, liver lipid does not correlate with muscle pH. Only liver glycogen does this (Love et al. 1974a), and a very rapid method for measuring the glycogen content at sea still needs to be designed. Small pieces of tissue can be sampled fairly accurately on a volume basis instead of weight (Love and Munro unpublished data), so glycogen determinations on a boat are not impossible.

Most of the information given in this review was obtained from investigations on cod; results on other commercial species are extremely sparse and it is desirable that similar work be done on them.

Acknowledgments

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Evaluation of the Transplant of Pacific Pink Salmon (*Oncorhynchus gorbuscha*) from British Columbia to Newfoundland

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LEAR, W. H. 1975. Evaluation of the transplant of Pacific pink salmon (*Oncorhynchus gorbuscha*) from British Columbia to Newfoundland. J. Fish. Res. Board Can. 32: 2343–2356.

Five transplants of eyed eggs of Pacific pink salmon (*Oncorhynchus gorbuscha*) were made between streams in southern British Columbia and North Harbour River, Newfoundland. Quantities transplanted were 0.25 million in 1959, 2.5 million in 1962, 3.4 million in 1964, 3.3 million in 1965, and 5.9 million in 1966. Adult returns from these transplants were 1, 49, 638, 8500, and 2426 during 1961, 1964, 1966, 1967, and 1968, respectively. Since 1969, returns have been the progeny of naturally spawning fish and have steadily declined in numbers. The reasons for failure of the transplant are not certain, but possibilities include the following: predation on the fry by brook trout and possibly eels in North Harbour Pond and estuary; unfavorable surface temperatures in the river during the fry run; predation by herring on fry in St. Mary's Bay; year-class failure of the even-year stocks that were introduced; unsuitability of the donor stocks with respect to migration patterns and homing behavior; inadequate numbers of eggs were transplanted to produce populations required to maintain runs in anything below optimum environmental conditions.

LEAR, W. H. 1975. Evaluation of the transplant of Pacific pink salmon (*Oncorhynchus gorbuscha*) from British Columbia to Newfoundland. J. Fish. Res. Board Can. 32: 2343–2356.

On a effectué cinq transplantations d'œufs embryonnés de saumon rose du Pacifique (*Oncorhynchus gorbuscha*) de cours d'eau du sud de la Colombie-Britannique dans la rivière Harbour-Nord, Terre-Neuve. Les quantités transplantées ont été de 0.25 million en 1959, 2.5 millions en 1962, 3.4 millions en 1964, 3.3 millions en 1965 et 5.9 millions en 1966. Les retours d'adultes résultant de ces transplantations ont été de 1, 49, 638, 8500 et 2426 en 1961, 1964, 1966, 1967 et 1968, respectivement. Depuis 1969, les retours sont ceux de la progéniture de poissons frayant naturellement, et leur nombre a diminué régulièrement. On n'est pas sûr des raisons de l'échec de ces transplantations, mais on mentionne les possibilités suivantes: prédation de l'omble de fontaine et possiblement de l'anguille sur les alevins dans l'étang Harbour-Nord et dans l'estuaire; températures de surface défavorables dans la rivière au moment de la migration des alevins; prédation du hareng sur les alevins dans la baie Ste-Marie; échec de la classe d'âge des stocks d'années paires introduits; inaptitude des stocks donneurs par rapport aux modalités de migration et au comportement de retour à la rivière d'origine; nombre d'œufs transplantés insuffisant à produire les populations requises au maintien de montaisons dans des conditions ambiantes moins qu'optimales.

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INTRODUCTION of Pacific pink salmon (*Oncorhynchus gorbuscha*) to the North Atlantic has been attempted since the early 1900s. Attempts to establish pink salmon in Maine from 1906 to 1926 were not successful although there were returns for a number of years (Bigelow and Schroeder 1953). During 1956, 513,000 pink salmon eggs and an additional 225,000 fingerlings were planted in Goose Creek, Hudson's Bay, but no adult pink salmon have been reported from Hudson Bay and its tributaries (Ricker and Loftus 1968). Transplantation of pink salmon from the western Pacific to the Barents Sea and White Sea was started in 1956 and initial results appeared promising (Kossov et al. 1960). Unfortunately, eggs deposited by the returning fish failed to produce fry in any quantity. Although fish of the second native generation spawned in the rivers in 1967, the 1969 runs were very poor (Ricker 1972). The great majority of the Kola Peninsula transplants returned to the general geographical region into which they were re-

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leased, but most failed to reach the river in which they had been hatched.

An introduction of pink salmon to Newfoundland was started in 1958. This paper presents a review of five transplants of eyed eggs made in the years 1959, 1962, 1964, 1965 and 1966, and an assessment of the resulting native generations; possible reasons for their decline in numbers are suggested.

Initial Planning and Development

This project was initially requested by the Newfoundland Wildlife Branch in a representation to the Salmon Co-ordinating Committee, and was sponsored by the Fisheries Research Board of Canada under the chairmanship of Dr. J. L. Kask. Much of the initial planning and development was done by Drs W. E. Ricker, A. A. Blair, and Mr W. P. Wickett in consultation with Dr W. Templeman, then Director of the St. John's Biological Station, and Dr A. W. H. Needler, then Director of the Nanaimo Biological Station.

Pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon were recommended for initial transplantation to eastern Canada because:

1) the primary objective was to establish a commercial fishery;

2) in fresh water these species required very little food in the case of chums or no food in the case of pinks, hence could not compete with native fish for food;

3) they were the most cold-tolerant of all five species of Pacific salmon and hence best able to cope with the cool waters that prevail off the Atlantic coast during much of the year.

Newfoundland was considered the best place for the initial introduction 1) because it was most in need of diversification of its fishing economy, 2) because it was centrally situated with respect to the part of the ocean most favorable for salmon, and 3) because it was not troubled with DDT spraying.

There was evidence that a salmon's ability to survive and return to its stream of origin in satisfactory numbers was partly hereditary. This being so, then the process of establishing a new run could be considered as a speeded-up process of adaptation by natural selection, with a consequent poor rate of return during the first few generations. This suggested three desirable characteristics for any program of transplantation:

1) Relatively large plantings should be made to one or a few neighboring sites, at first, so that there would be an adequate expendable surplus while the selection process was weeding out genes that were maladaptive to the new situation. From the point of view of the stream to which the transplant was to be made, a million was a small number, and 10 million was certainly not large. If the aim were to be a seeding equivalent to a moderate-size run in a moderate-size stream, 15–20 million eggs would be needed — equivalent to 10,000–13,300 female pinks.

2) Donor stocks should be selected carefully in order to match up the freshwater and marine conditions of existence of the old and the new sites as closely as possible. Because the sea is cool around the new site, other fish from more northerly portions of the Pacific would probably take hold most quickly. On the other hand, if the latitudes of the two sites must be matched because the fish may navigate in the ocean due to an innate response to incident light, then the most southerly stocks should be chosen as donors because the south shore of Newfoundland is at 47°N.

Another possibility was that most specimens of an east-Pacific stock would tend to migrate east as they approach maturity, hence a donor stock from e.g. southern Kamchatka would be a more suitable donor than any American stock.

3) The influence of the environment: fluctuations in the stock from less than the spawning population to more than 20 times the number of spawners can be caused by the environment. Consistently high-producing streams have a low gradient, and loose clean gravel that is not subject to scouring or drying. Colder and lower salinity conditions in the sea when the fry migrate from the streams are related to poorer ocean survival. Incubation temperatures below 4 C during the pre-eyed stage, i.e. up to 200 degree-days, cause high mortality. The numbers of predators in the stream and estuary have a bearing on the minimum number of fry that produces a self-sustaining stock.

To make the best use of transplanted eggs, provision must be made to provide optimum fresh water conditions in the initial stages of the project so as to guarantee high survival.

The general requirements of pink and chum salmon are shown in Table 1.

It was finally recommended that the donor streams would be one or more of the Tsolum, Oyster, Koeye or Lakelse rivers, and that pink salmon eggs were to be used for the first 3 yr at least since these would give answers on returns most quickly.

The project was to be in two phases:

1) Experimental phase in 1959–60 would consist of transplanting up to 1,000,000 eggs to

TABLE 1. General requirements of pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon.

	O. gorbuscha		*O. keta*	
	Fry	Adults	Fry	Adults
Stream: temperature	2–8 C	8–12 C	2–8 C	6–10 C
gradient	0.001–0.01	0.001–0.01	0.001–0.01	0.001–0.01
flow	even flow	0.2–0.6 m/s	even flow	0.2–0.6 m/s
depth	0.2–0.5 m	0.2–0.5 m	0.2–0.5 m	0.2–0.5 m
substrate	loose, clean gravel 1.3–5.0 cm	loose, clean gravel 1.3–5.0 cm	loose, clean gravel 1.3–5.0 cm	loose, clean gravel 1.3–5.0 cm
Ocean: temperature	4–12 C	4–15 C	4–12 C	4–15 C
salinity (coastal)	32‰	32‰	32‰	32‰

check on freshwater survival more fully, and to familiarize personnel with shipping, planting, and incubation problems.

2) Pilot plant phase in 1960–61 would be the first real test of sea survival. It was recommended that 10–25 million eggs would be transplanted to one stream for each of these 2 yr. The results would be first seen in 1962 (W. P. Wickett, personal communication).

Transplant Site

During June 26–July 24, 1958, Dr A. A. Blair and Mr W. P. Wickett visited 14 streams in Newfoundland. Streams were rated for gravel, waterflow, presence of side channels for planting and incubation, public relations, presence of predators, and ease of transportation. Four streams in St. Mary's Bay on the Avalon Peninsula were well suited to the purpose. North Harbour River at the head of St. Mary's Bay was recommended for the first transplant on the basis that it met the above-mentioned criteria, and that the ocean and freshwater properties (Table 1) appeared favorable. It was not too far removed from the St. John's Biological Station and the airport, and it presented a minimum of interference to native salmonids, because the stock of Atlantic salmon (*Salmo salar*) in this river was very low.

Two controlled flow spawning channels were used for the egg plantings. Stump Channel, which was used for the 1959 egg planting, was 111 meters long of which 84 meters were gravelled, and 3 meters wide. Herder's Channel was used for all egg plantings after 1959 and was also the spawning site for most of the naturally spawning fish that returned to the river; it was 540 meters long overall with 480 meters of gravelled area, and 5 meters wide.

Methods

Egg Collection and Transportation

Eggs were collected from mature pink salmon on the spawning grounds of three British Columbia streams (see Table 4) and where shipped to Newfoundland by air. The 1959 eggs were taken from the Indian River which runs into the upper end of Burrard Inlet, near Vancouver. They were transported green by river boat, launch, truck, and ferry to the Puntledge Hatchery at Courtenay, Vancouver Island in 8 h on September 26, October 3, 10, and 17. After eyeing, they were flown from Courtenay to St. John's via Winnipeg, Montreal, Moncton, Halifax, Sydney, Stephenville, and Gander. A similar procedure was used in subsequent years, though float planes were used to bring eggs in from Glendale Creek on Knight Inlet where a large collecting installation was located by the Fish Culture Branch (W. P. Wickett, personal communication).

The eyed eggs were shipped to Newfoundland by air in insulated cases with a capacity of 100,000 eggs. Two types of shipping case were used, one constructed of a wooden frame with fiberglass insulation, and the other made entirely of styrofoam. The latter type was preferred because of its lighter weight. The eggs were wrapped in cheese-cloth, placed in trays on a layer of damp moss, and kept moist with water from melting ice above the eggs. The eggs were trucked 105 km from St. John's Airport to North Harbour River in St. Mary's Bay on the south coast of Newfoundland (Fig. 1).

Egg Planting

All eggs were planted in North Harbour River in two controlled flow channels, Stump and Herder's, 0.8 km and 4.0 km from the river mouth, respectively (Fig. 1). Because of heavy silting the Stump Channel was used only in the first transplant in 1959. Eggs were measured volumetrically and the actual number in every tenth measure was determined by using a Swedish egg counter (Lindroth 1956). The eggs were

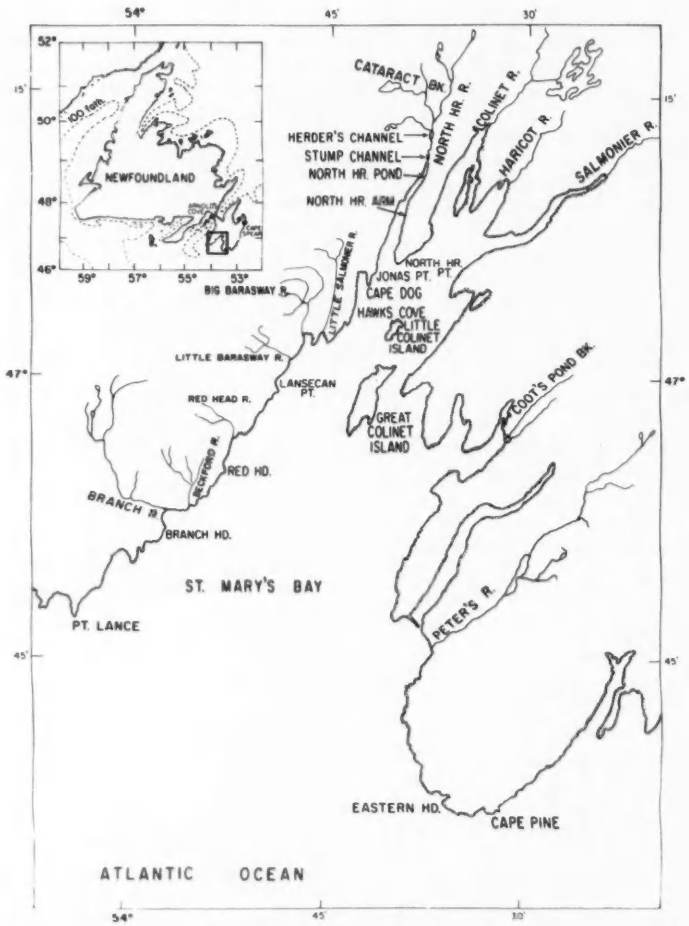

FIG. 1. St. Mary's Bay, Newfoundland, showing place names mentioned in the text.

planted 20 cm deep in trenches dug across the channel. The eggs were poured into a trench from an insulated bucket and covered with gravel removed in digging the next trench located 50 cm downstream. During planting the flow of water was reduced so that the depth of water was about 3 cm over the gravel. The density of planting was 1900–4300 eggs/m² (Blair 1968). The channels contained screened gravel to a depth of 30 cm. Gravel sizes were 1–5 cm in diameter in Stump Channel and 2–7.5 cm in Herder's Channel.

FRY COUNT

The pink salmon fry migrate downstream immediately after they emerge from the gravel at which time they have attained a length of 25–35 mm.

In the egg channels the numbers of fry were estimated by weighing in water in 200-g lots and by actual count of the number in each tenth weighing. The trapping device in Stump Channel in 1960 consisted of a fence of wooden framework covered with 2.5-cm mesh and 3-mm nylon netting with two funnels leading into small removable traps (30 cm × 30 cm × 90 cm) of similar construction. The same type of weir was used in Herder's Channel in 1963 but with one additional trap. This type of trap was unsatisfactory for large numbers of fry because it was difficult to clear the trap fast enough to keep the fry from suffocating. A Wolf trap (Wolf 1951) was used in Herder's Channel in 1965 and proved satisfactory. During 1960, 1963, and 1965 actual counts of fry were obtained by this method. To avoid handling the fry, in 1966 and 1967, migrants

were estimated by applying the rate of survival of eggs to the fry stage in perforated aluminum or plastic containers (25 eggs each) which were buried in the gravel of every tenth trench in the spawning channel and which were dug out and checked when fry were emerging the following spring. A similar method was also used in the main river during 1968–72 as a check against a mark–recapture method. Since 1969 the total numbers of fry migrants in the main river have been estimated using the mark and recapture method and the modified formula suggested by Bailey (1951). Since 1969 individual fry in the channel were counted when they were captured in the Wolf trap.

Fry Movements

Visual searching for fry in North Harbour Pond, North Harbour Arm, and around St. Mary's Bay was conducted from the middle of May to the end of July whenever weather permitted, and at irregular intervals after July. Numbers of fry seen were estimated. Observations in North Harbour Pond and estuary were made along the shore and near the sand bars at low tide. In North Harbour Arm and St. Mary's Bay a motor boat and row boat were used to search along the shore and in the small coves. A small nylon beach seine was used for sampling juveniles.

Fry Predation in North Harbour Arm

During 1963 and 1965–68 two fleets of standard gillnets were fished in North Harbour Arm during May–June to catch samples of fish in the area to determine if there was predation on pink salmon fry. A fleet consisted of six sections of netting each of which was 23 meters long. The mesh sizes of the sections were 38, 51, 76, 102, 127, and 140 mm between the knots when meshes were stretched. During 1963, 1965–68, and 1970 representative samples of Atlantic salmon smolts, trout, eels, and smelt migrating downstream through the counting trap were taken and their stomachs examined for pink salmon fry.

Table 2. Dates of entire runs, 75% modes of runs, and peak week of runs of pink salmon fry in North Harbour River during 1960, 1963, and 1965–73.

Year	Entire run	75% of run	Peak week
1960	Apr. 11–May 31	May 12–18	May 15–21
1963	May 7–June 16	May 21–25	May 19–25
1965	Apr. 29–June 19	May 26–June 5	May 30–June 5
1966	Apr. 30–May 31	May 16–23	May 15–21
1967	Apr. 30–May 31	May 16–23	May 14–20
1968	Apr. 2–May 23	May 5–11	May 5–11
1969	Apr. 16–May 16	Apr. 20–May 3	Apr. 27–May 3
1970	Apr. 9–May 10	Apr. 24–27	Apr. 26–May 2
1971	Apr. 14–May 5	Apr. 22–27	Apr. 25–May 1
1972	Apr. 13–May 18	May 2–6	Apr. 30–May 6
1973	Apr. 19–May 18	May 6–10	May 6–12

Table 3. Means and standard deviations of surface water temperatures (C) during entire fry run, 75% mode, and peak week of fry run in North Harbour River and during entire fry run and 1 mo after fry run in North Harbour Arm.

	North Harbour River						North Harbour Arm			
	Entire run		75% mode		Peek week		Entire run		Month after run	
Year	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1960	8.66	4.82	12.55	2.18	11.64	1.89	6.30	1.62	10.04	1.67
1963	9.29	3.98	11.35	3.14	11.14	3.13	5.50	0.71	10.11	1.00
1965	10.85	3.18	10.33	2.22	10.70	1.69	5.34	2.21	10.38	2.55
1966	8.58	3.49	9.38	2.55	8.64	2.24	6.58	1.78	8.00	1.98
1967	7.55	2.89	8.94	2.42	8.41	2.67	4.07	1.56	7.35	3.04
1968	6.51	3.13	7.43	1.11	7.43	1.11	3.70	1.15	7.19	1.64
1969	7.54	1.85	6.71	1.31	7.55	0.66	3.13	1.32	6.56	1.75
1970	6.57	3.34	8.09	3.33	8.32	2.67	5.19	1.26	7.03	0.88
1971	7.80	2.06	7.77	1.03	6.26	1.71	5.02	0.75	8.54	1.88
1972	6.23	2.20	6.15	1.78	6.21	1.72	2.78	1.05	7.85	3.40
1973	7.55	2.19	6.86	1.26	7.73	2.05	2.51	1.13	5.92	2.00

TABLE 4. Transplants of eyed eggs of pink salmon from British Columbia to North Harbour River, Nfld. during 1959, 1960, and 1964–66, eggs deposited by natural spawning fish during 1966–73, subsequent fry migrants, and adult returns.

	Eggs		Fry migrants			Adult returns			British Columbia		Newfoundland	
									Donor		Egg Channel	
Transplant no.	Year	No. millions	Year	No. millions	% of eggs	Year	North Hr. R.	Total	Stream	Lat.	North Hr. R.	Lat.
1	1959*	0.25	1960	0.10	38	1961	1	1	Indian R.	49° N	Stump	47° N
2	1962*	2.5	1963	2.15	87	1964	25	49	Glendale R.	51° N	Herder's	47° N
3	1964*	3.4	1965	2.86	83	1966	419	638	Lakelse R.	54° N	Herder's	47° N
		(0.02)										
4	1965*	3.3	1966	3.00	91	1967	5334	8500	Lakelse R.		Herder's	47° N
5	1966	5.9*	1967	5.10	82	1968	1353	2426	Lakelse R.		Herder's	47° N
		(0.5)										
	1967	(4.4)	1968	3.80	87	1969	1116	2603				
	1968	(1.1)	1969	0.86	76	1970	1489	2091				
	1969	(0.9)	1970	0.67	72	1971	468	624				
	1970	(1.2)	1971	0.87	70	1972	58	117				
	1971	(0.4)	1972	0.27	72	1973	60	174				
	1972	(0.05)	1973	0.04	79	1974	18	28				
	1973	(0.05)	1974	0.04	71							
	1974	(0.014)	1975	0.010	67							

*Transplanted; ( ) deposited naturally.

Adult Count

Adult pink salmon entering North Harbour River were counted at a fence 366 meters upstream from the river mouth. The fence was similar to one described by Blair (1957). In nearby rivers periodic visual searches were made for pink salmon. In other rivers reports of sightings or captures were verified and compiled. Since 1966 commercial fishermen in St. Mary's Bay recorded pink salmon catches on provided forms. In other areas during 1966–70 fishermen were interviewed to verify reports of catches. Beginning in 1971 forms have been distributed to fisheries officers and wardens around Newfoundland and these are returned each autumn giving details of any pink salmon taken by the commercial and angling fisheries or observed in rivers other than North Harbour River.

When available, samples of adults from the commercial fishery and the rivers were obtained and examined for length, weight, and sex.

Hydrographic Data

Water temperatures were recorded by a Negretti and Sambra continuous temperature recorder with a weekly time scale during 1958–67 and by a Ryan recorder with a 30-day scale since 1967. The averages and standard deviations of the water temperatures in North Harbour River during the entire fry run, the 75% mode and the peak week of the run were calculated as well as the temperature in North Harbour Arm during the fry run and the month after the fry run (Tables 2 and 3).

Results

Egg to Fry Survivals

During the period November 24–27, 1959, 0.25 million eggs collected from Indian River, British Columbia, were planted in Stump Channel, North Harbour River (Table 4). The winter of 1959–60 was fairly mild and the channel was iced over only from December 20 to the last week in February. The estimated number of fry migrating from the channel in 1960 was 0.1 million (38%). This low rate of survival was thought to be due to excessive silting in Stump Channel so this channel was not used again for incubating eggs. Eighty fry were seen at the mouth of the river on May 23 and 24. On five different occasions from May 24 to July 3 fishermen reported seeing small fish presumed to be pink fry in North Harbour Pond and Arm.

The November 22–December 3, 1962 planting of 2.5 million eggs from Glendale River into Herder's Channel produced an estimated 2.2 million fry in 1963 for a survival rate of 87%. Fry were seen by Station personnel in the mouth of the river from May 30 to June 4, in the pond from May 20 to July 7, in the arm from June 24 to July 9, and in the bay from July 2 to July 31. The total number of fry seen in 30 sightings was estimated to be over 27,000. The last group was seen on July 31 in St. Mary's Bay near Red Head about 22 miles from the mouth of the river.

The 1964 transfer and planting of 3.4 million eggs took place between January 12 and 25, 1965, which was late in comparison with other planting times. This was because the eggs were slow in developing. On trial shipments there was a 40% mortality on November 21 and 0.7% on December 17, whereas in the transfers in January it was 0.2%. This low value is similar to that observed in previous transfers. There were an estimated 2.9 million fry leaving the channel in 1965, or 83% of the eggs planted. Observations indicated a rapid movement of fry from the river to the bay. They were seen in the lower reaches of the river May 23–27, in the pond May 24–June 18, and in the bay June 8–July 13. The total number seen in 49 sightings was estimated at 63,000. The last group was seen July 13 about 30 miles from the river.

During November 25 to December 3, 1965, 3.3 million eggs were planted. The estimated number of fry leaving the channel in 1966 was 3.0 million or 91% of the eggs planted. They were seen in the mouth of the river from May 9 to 23, in the pond from May 16 to June 11, in the arm from May 25 to June 3, and in the bay from May 26 to June 28. The estimated number in 55 sightings was 138,000. The numbers sighted are not truly reflective of the size of the fry run in St. Mary's Bay since these observations were conducted mainly to confirm that fry were surviving the estuarine migration and were actually moving out to sea.

The fifth and final transplant consisted of 5.9 million eggs planted in Herder's Channel during November 1–12, 1966. The estimated number of fry leaving the channel based upon survival rates in egg containers planted in the gravel, was 4.8 million or 82% survival. In addition there were an esimated 0.3 million fry from the 1966 spawning of 638 adults in the river, giving a total fry run of 5.1 million. Fry were seen in the mouth of the river during May 17–23, in the pond during May 17 to June 8, in the arm during May 19 to June 8, and in the bay from May 31 to July 17. Total fry seen in 38 observations was 83,000.

Egg deposition since 1966 has been entirely from fish spawning naturally. The estimated egg deposition, based on 1600 eggs per female fish, ranged from 4.4 million in 1967 to 0.014 million in 1974. The egg to fry survival rate estimated on the basis of samples placed in the river prior

TABLE 5. Predation on pink salmon fry in North Harbour River by brook trout, brown trout, Atlantic salmon smolts, eels, and smelt during 1963, 1965–68, and 1970.

Species	No. of fish examined	No. of fish with fry	No. of fry	Avg. no. of fry/fish
Brook trout	1067	87	1091	1.02
Brown trout	332	25	95	0.29
Smolts	100	5	10	0.10
Eels	173	3	4	0.02
Smelt	310	1	1	<0.01

to 1969 and by the mark–recapture method since 1969, ranged from 87% in 1968 to 67% in 1975 (Table 4).

FRY PREDATION

During 1963, 1965–68, and 1970 stomach contents of samples of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*) smolts, American eels (*Anguilla rostrata*), and rainbow smelt (*Osmerus mordax*) migrating downstream in North Harbour River revealed that brook trout were feeding more heavily (1 fry per fish) than the other species on pink salmon fry (Table 5). Brown trout contained 0.29 fry per fish examined, while smolts, eels, and smelt were feeding to a minor degree on pink salmon fry. It is suspected that pink fry, on the basis of their size and behavior, would almost certainly constitute an ideal prey for eels. The predation by eels could be grossly underestimated since eels feed mainly at night while the eel stomachs were collected at the counting trap and examined during daylight hours.

During 1963 and 1965–68 a total of 9688 fish were taken by gillnets in North Harbour Arm. Species caught, in descending order of numbers were cunner (*Tautogolabrus adspersus*), Atlantic herring (*Clupea harengus harengus*), winter flounder (*Pseudopleuronectes americanus*), shorthorn sculpin (*Myoxocephalus scorpius*), rainbow smelt (*Osmerus mordax*), Atlantic cod (*Gadus morhua*), thorny skate (*Raja radiata*), brook trout (*Salvelinus fontinalis*), Atlantic tomcod (*Microgadus tomcod*), Atlantic mackerel (*Scomber scombrus*), brown trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), and Northern wolffish (*Anarhichas denticulatus*). Pink salmon fry were found in stomachs of 20 of the 9688 fish examined. The species feeding on the pink fry were 15 brook trout, which ate a total of 26 fry, 2 sculpins (2 fry); 1 cunner, 1 cod, and 1 herring each had 1 fry in their stomach contents.

In addition 89 cod and 19 Atlantic salmon taken by commercial fishermen in the North Harbour area were examined but none had fry in their stomachs. Also 1010 capelin (*Mallotus villosus*) seined in North Harbour Arm during the spawning period in June were examined and none contained any fry. It appears that the most serious predator of pink salmon fry are the brook trout in the river and the estuary.

FRY DISTRIBUTION

During the years when regular searching was conducted for fry in St. Mary's Bay, the pink salmon fry were observed to leave North Harbour Arm and move along the western side of the bay (Fig. 2). The fry were observed close to shore and frequented tiny coves and inlets with heavy seaweed growths.

DISTRIBUTION OF JUVENILES

Juveniles also were mainly observed on the western side of St. Mary's Bay (Fig. 3). Samples of these were obtained by dipnet and beach seine during 1966 and 1967. There were reports of juveniles at Arnold's Cove in nearby Placentia Bay during August–October, 1966. A few of these were caught from the community wharf on a rod by fly and bait, and measured at the Biological Station. The average fork lengths of pink salmon juveniles sampled during 1966–67 were as follows: June 16, 1966 — 45.1 mm (25 fish), June 29, 1967 — 38.2 mm (6), July 5, 1967 — 52.0 mm (2), July 17, 1967 — 60.0 mm (25), August 21, 1966 — 135.0 mm (3), October 5, 1966 — 188.2 mm (5), October 12, 1967 — 180.3 mm (14), October 20, 1966 — 192.0 mm (1), and October 25, 1966 — 213.0 mm (9).

ADULT RETURNS

There was only one observed adult return to North Harbour River from the 1959 egg trans-

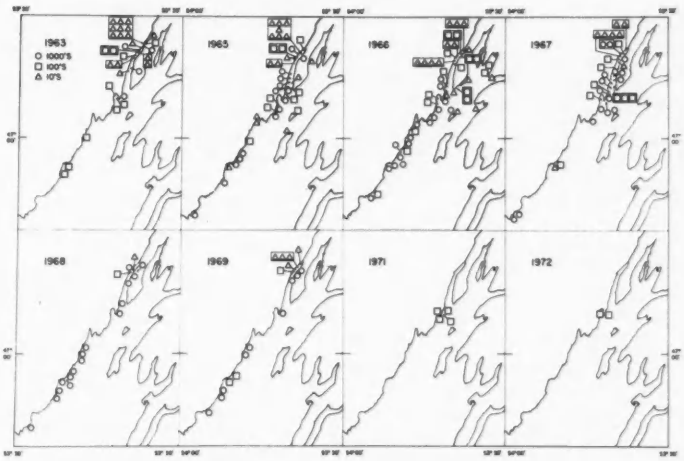

FIG. 2. Distribution and abundance of pink salmon (*Oncorhynchus gorbuscha*) fry in St. Mary's Bay during 1963, 1965–69, and 1971–72.

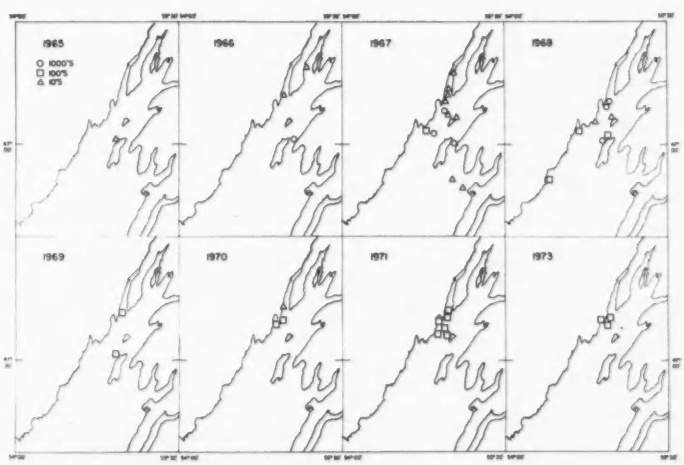

FIG. 3. Distribution and abundance of juvenile pink salmon in St. Mary's Bay during 1965–71 and 1973.

plant. From the 1962 transplant there were 49 adult returns of which 25 returned to North Harbour River, the remainder being taken in St. Mary's Bay (Fig. 4).

There was a total of 638 adult returns from the 1964 egg transplant, all taken in St. Mary's Bay or in rivers flowing into that bay; 419 of these were recorded in North Harbour River (Fig. 4). From the 1965 transplant of 3.3 million eggs there were 8500 returns; 5334 were observed in North Harbour River, 1187 went to the commercial fishery in St. Mary's Bay, 34 to two other rivers in St. Mary's Bay, and the remainder to coastal fisheries and streams mainly along the northeast coast of Newfoundland (Fig. 4). There were three returns from Nova Scotia and two from Quebec. This large return during 1967 was produced by approximately the same numbers of eggs and fry that produced only 638 returns in 1966 (Table 4).

From the largest transplant of 5.9 million eggs in 1966, there were 2426 adult returns in 1968;

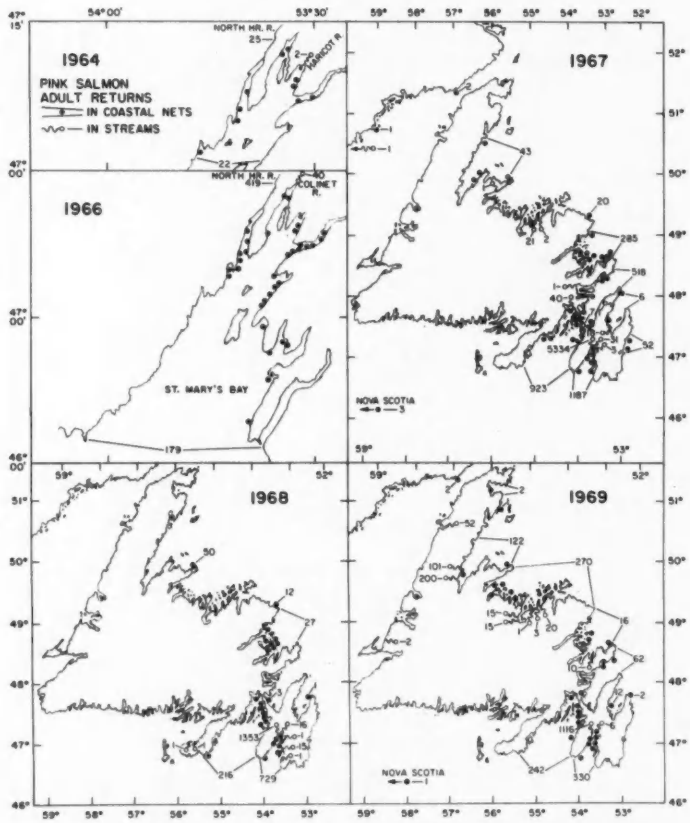

FIG. 4. Distribution of adult pink salmon during 1964, 1966–69 in North Harbour River, in coastal nets, and other streams.

1353 were from North Harbour River, 762 from St. Mary's Bay, and the remainder from the northeast coast of Newfoundland (Fig. 4).

The 1969 return of 2603 adults was produced from the natural spawning of 5334 parents during 1967. This is a substantial decrease in numbers from the parent stock. The returns outside St. Mary's Bay were from the northeast and northwest coasts of Newfoundland (Fig. 4). This return produced the highest number of pink salmon adults taken outside North Harbour River (Fig. 5). The rate of recapture outside St. Mary's Bay during other years was less than 25% except for the 1969 return which had a rate of 44% (Fig. 6). The 1970 run of 2091, of which 1489 returned to North Harbour River, was produced by 1353 parents which had returned to the river in 1968 (Fig. 7). Since 1970 the runs have been decreasing sharply. In 1972, 1973, and 1974 the returns to North Harbour River were 58, 60, and 18, respectively (Table 4). During 1973, there were six verified reports of adult pink salmon taken in northern Labrador, of which two were taken in river systems, the other four in coastal fisheries.

LENGTH DISTRIBUTIONS

Fork lengths were obtained from samples of adult pink salmon captured by commercial fishermen in St. Mary's Bay during 1964 and 1966–73. Analyses of variance revealed that annual differences in fork lengths were significant at less than the 0.001 level for both the even year ($F_{4283} = 10.74$) and the odd year ($F_{3359} = 8.56$) cycles. There was a trend towards decreasing fork length from 1969 to 1973 in both the odd and even year cycles (Fig. 8). Overall average fork length of

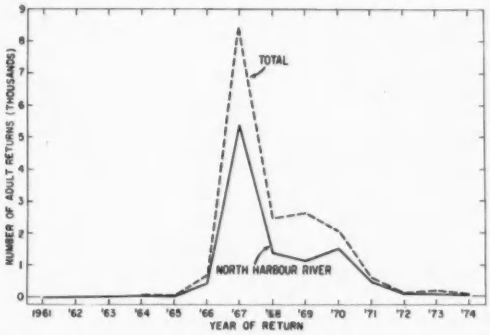

FIG. 5. Annual returns of pink salmon adults to North Harbour River (solid line) and total returns to all areas (broken line), 1961–74.

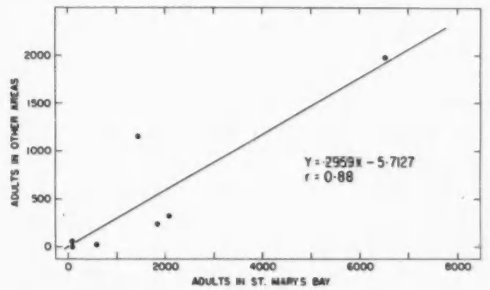

FIG. 6. Returns of pink salmon to St. Mary's Bay plotted against those reported from other areas during 1967–73.

pink salmon of the odd year cycle (54.98 cm) was significantly greater ($P < .001$) than that of the even year cycle (49.22 cm). The average fork lengths were regressed against the sea surface temperatures in the southeastern Newfoundland area during the entire life span of each pink salmon run of the odd and even year cycles as well as the monthly and combined monthly averages during winter, spring, and summer of the return year. No significant correlations existed between the average fork lengths of pink salmon of the odd year cycle and the sea surface temperatures. A significant correlation ($r = 0.90$, $.02 < P < .05$) was found between the average fork lengths of pink salmon of the even year cycle and the average sea surface temperature at station 27 during January–August of the return year; linear regression of fork length on temperature was $Y = 5.3709X + 35.8039$.

POSSIBLE CAUSES OF DECLINE IN NUMBERS

In the Pacific it has been reported by Neave (1953) and Hunter (1959) that the marine mortality of pink salmon fry, exclusive of fishing, is approximately 95%, although severe departures from this figure also have been observed.

Vernon (1958) reported that the best single factor for predicting the rate of return of Fraser River pink salmon appeared to be the mean April to August seawater temperature in Georgia Strait. There was an inverse relationship between the mean temperature and the subsequent survival rate. Vernon postulated that the seawater temperature possibly acted indirectly through the food supply or the distribution of predators, or through the distribution of young pink salmon in relation to these factors. In an attempt to demonstrate this relationship with the transplanted fish, the survival rates of pink salmon per 1000 migrating fry of the odd and even year cycles, and combined cycles of the 1964–71 year-classes, were fitted by a least-squares regression against the average surface temperatures in North Harbour River during the entire fry run, the peak week of the run, and the 75% mode of the run. Also, the survival rates of the odd and even year cycles, and combined cycles of the 1964–71 year-classes, were fitted to the average surface temperatures in North Harbour Arm during the entire fry run and during the month after the fry run. Additionally these survival rates were fitted to the average seawater temperatures at the surface and at 25 meters during each of the months of June and July at station 27 near Cape Spear (Fig. 1). The only significant correlation that was found in all the preceding regressions was that between the survival rate of the odd year cycle (1965–71 year-classes) and the surface temperature in North Harbour River averaged over the entire fry run. The linear regression $Y = 0.9607X - 5.4232$ where Y = survival rate (adults/1000 fry) and X = surface temperature (C) had a correlation coefficient of 0.99 ($P < .01$) (Table 6). Thus the surface temperature in the river during the fry run may be implicated as a possible cause of fry mortality when one considers that the average temperatures during the fry run have decreased significantly ($P < .001$) since 1965 (Table 3).

Henry (1961) obtained a correlation between first-year ocean growth (as indicated by number of scale circuli) and percentage sea survival of pink salmon from the Fraser River, B.C. He reported that an 11% increase in numbers of circuli corresponded with a 22% increase in survival. The survival rates of pink salmon of the even, odd, and combined cycles of the 1962,

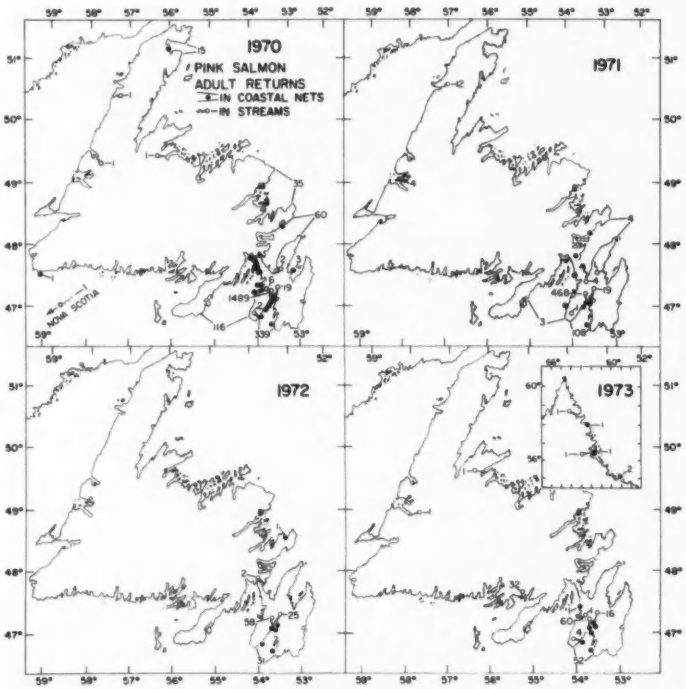

FIG. 7. Distribution of pink salmon adults during 1970–73 in North Harbour River, in coastal nets and other streams.

1964–69 year-classes were plotted against all, and part of, the first-year ocean growth using the numbers of circuli in the entire first-sea year, the number in the first half of the first-sea year, and the number in the second half of the first-sea year. There was no significant correlation between any of the indices of first-year growth and adult survivals of either the even, odd, or combined cycles.

Hunter (1959) found that predation was important in reducing the numbers of fry. Vernon (1958) stated that in southeastern Alaska, herring were feeding on young pink salmon and attributed the fluctuations of Fraser River pink salmon to variable predation by the herring populations in Georgia Strait. On the premise that predation by herring and mackerel might account for variation in return of pink salmon, and as-

TABLE 6. Basic data points to which regression equations were fitted.

Year-class	Adults/1000 fry	Mean fork length (*cm*)	Sea temp (C) Jan.–Aug. (Sta 27)	Temp (C) North Harbour River (fry run)	Herring stock sizes (*tons*)
1962	0.02	46.75	1.81	9.29	—
1964	0.22	48.88	2.46	10.85	38,000
1965	2.83	55.35	2.19	8.58	42,000
1966	0.48	48.58	2.68	7.55	41,000
1967	0.69	55.28	2.79	6.51	59,200
1968	2.43	55.16	3.32	7.54	20,000
1969	0.93	54.18	3.20	6.57	35,200
1970	0.14	48.88	2.62	7.80	40,500
1971	0.65	49.65	2.00	6.23	41,200

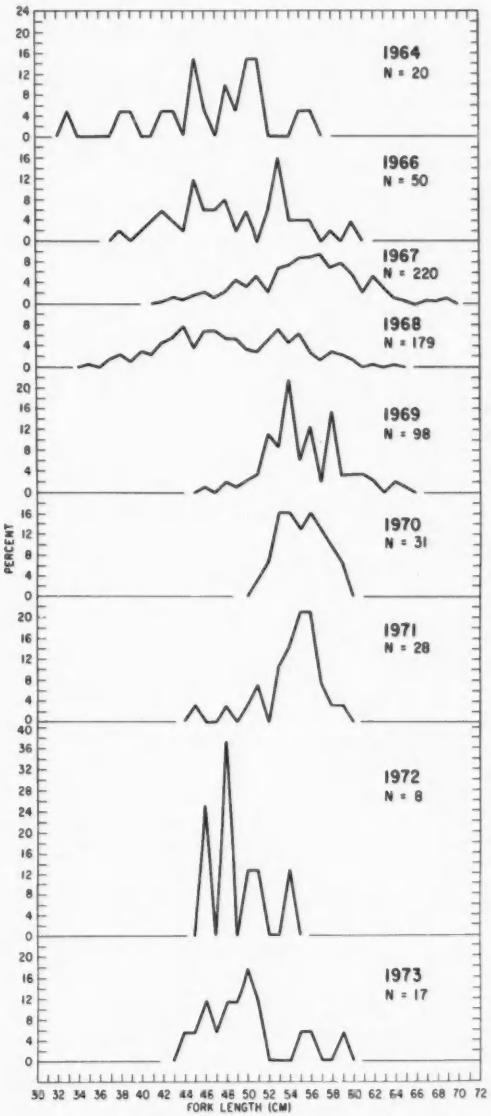

FIG. 8. Length distribution of adult pink salmon caught in commercial nets and taken in North Harbour River during 1964, 1966–73.

suming that the predation rate would be proportional to stock sizes of herring and mackerel in the area, the survival rates of pink salmon, of the even, odd, and combined year cycles of the 1964–71 year-classes were plotted against the herring stock sizes calculated by virtual population analyses (G. H. Winters, personal communication) in the St. Mary's Bay-Placentia Bay area, and also against the indices of mackerel abundance in St. Mary's Bay (G. H. Winters, personal communication). Although no significant correlation was found between mackerel stock sizes and the return rate of pink salmon, there was a significant inverse correlation ($r = 0.97$, $P < .05$) between the survival rates of pink salmon of the even year cycle (1964–70 year-classes) and the herring stock sizes in the St. Mary's–Placentia Bay area. The linear relationship is $Y = -0.1057X + 4.5040$ where Y = number of adults/1000 fry and X = herring stock size (thousands of metric tons) (Table 6).

Aro and Shepard (1967) report that in northern and central British Columbia, pink salmon are abundant in both even and odd year lines, but in some areas either the even or odd year stock vastly outnumbers that of the opposite line. In some rivers there have been shifts in dominance between even and odd year cycles. Of the four largest egg transplants three were of even year stocks from southern British Columbia. The transplant that produced the largest returns (almost 4 times the next largest) was the odd year stock of 1965. Thus the use of even year stocks could also be a contributory factor to the lack of success of the transplant.

It appeared that the most serious predator on the pink salmon fry both in the river and in the estuary were the brook trout. It also is suspected that the predation rate in North Harbour Pond, between the river and the estuary, was higher than in the river or estuary since the brook trout do not migrate directly to sea but inhabit the pond for periods varying from a few days to several weeks. In such an enclosed area the predation pressures on the fry may be considerable. In view of the pattern of distribution and migration of the pink salmon fry along the west side of St. Mary's Bay during the summer, a high predation rate by resident and anadromous brook trout near Big Barasway River, Little Barasway River, Red Head River, Beckford's River, and Branch River, is highly possible. It is postulated that a significant portion of the sea mortality can be attributed to brook trout predation, although it is quite possible that eels may also be quite significant predators.

In conclusion, although the transplant to North Harbour River is an apparent failure, this does not necessarily mean that further transplants of pink salmon to the Atlantic would not be worthwhile. In the first place, the level of transplanting envisaged (10–25 million eggs annually for 2 consecutive yr) was not achieved and this may be a significant reason for the failure. With such low egg numbers the population may not be able

to recover from years of low marine survivals. Secondly, eggs were taken from the eastern Pacific and planted in the western Atlantic. Better results might have been obtained from eggs from the western Pacific since the migration patterns of western Pacific fish might be more compatible with current systems in the northwest Atlantic. Finally, subsequent distribution of pinks prior to the spawning period suggests that a river on the northeast coast of Newfoundland rather than the south coast may have proven more successful.

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I am particularly indebted to the late Dr A. A. Blair who conducted and coordinated the transplant operation at North Harbour River from 1958 until 1969. Mr F. A. Day was the senior technician on the transplant site and supervised most of the technical routines associated with the transplant and the collection of environmental data. Dr A. W. May was in charge of the project during 1969–71. Much of the data describing materials and methods, especially the egg transportation, planting, and fry counts were drawn freely from annual reports, field reports, and unpublished manuscript reports.

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Sensitivity of Blood Cell Counts in Juvenile Coho Salmon (*Oncorhynchus kisutch*) to Stressors Including Sublethal Concentrations of Pulpmill Effluent and Zinc

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McLEAY, D. J. 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulpmill effluent and zinc. J. Fish. Res. Board Can. 32: 2357–2364.

Transfer of fish from optimal to high-temperature, crowded holding conditions decreased white blood cell-thrombocyte (WBC-T) counts markedly on 12–96-h exposure. Red blood cell (RBC) counts were increased only slightly at 12 and 48 h. During 28 days acclimatization following transfer to simulated stream compartments, WBC-T counts returned to stock values within 2–4 days, following an initial decline. RBC counts were not altered appreciably.

The decline in WBC-T counts of acclimatized fish to a high sublethal concentration of pulpmill effluent was greatest at 24 h. Mean RBC counts were decreased over 24–96 h, but differences were significant at 24 h only. In acclimatized fish exposed to pulpmill effluent, WBC-T counts were decreased ($P < 0.001$) at concentrations between 0.2 and 0.9 of the 96-h LC50. A 24-h exposure to 15% vol/vol of this effluent had considerably less effect on the WBC-T count when the effluent had been detoxified by foam fractionation.

Under similar experimental conditions, exposure to sublethal levels of zinc depressed mean WBC-T counts proportional to concentration, although differences were only significant at 0.5 LC50 and greater. RBC counts were unchanged.

The WBC-T response provides a reasonably rapid and sensitive method for measuring stressful levels of pulpmill effluents to salmon. Declines in WBC-T counts are attributable to reduced numbers of circulating small lymphocytes, which could result in decreased resistance of stressed fishes to disease.

McLEAY, D. J. 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stressors including sublethal concentrations of pulpmill effluent and zinc. J. Fish. Res. Board Can. 32: 2357–2364.

Le transfert de poissons de conditions optimales à des conditions de surpeuplement et de haute température a pour effet de réduire notablement le compte de globules blancs-thrombocytes (GB-T) après exposition de 12-96 h. Les comptes de globules rouges (GR) n'augmentent que légèrement; à 12 et 48 h. Durant une période d'acclimatation de 28 jours après transfert dans des compartiments simulant un cours d'eau, les comptes de GB-T retournent aux valeurs des poissons de réserve en dedans de 2–4 jours, après un déclin initial.

Le déclin des comptes de GB-T des poissons acclimatés à une forte concentration sublétale d'effluent de papeterie est à son maximum à 24 h. Les comptes moyens de GR diminuent sur une période de 24–96 h, mais les différences ne sont significatives qu'à 24 h. Chez des poissons acclimatés exposés à l'effluent de papeterie, les comptes de GB-T diminuent ($P < 0.001$) à des concentrations variant entre 0.2 et 0.9 de la CL50 après 96 h. Une exposition de 24 h à 15% vol/vol de cet effluent a beaucoup moins d'effet sur le compte de GB-T après que l'effluent a été désintoxiqué par fractionnement de mousses.

Dans des conditions expérimentales semblables, l'exposition à des niveaux de zinc abaisse les comptes moyens de GB-T en proportion de la concentration, bien que les différences ne soient significatives qu'à 0.5 ou plus de la CL50. Les comptes de GR ne varient pas.

La réponse des GB-T donne une méthode raisonnablement rapide et sensible de mesure des niveaux d'effluent causant un stress chez le saumon. Le déclin des comptes de GB-T est attribuable à la diminution de petits lymphocytes en circulation. Ceci pourrait entrainer une diminution de la résistance à la maladie chez les poissons soumis à un stress.

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NUMBERS of circulating white blood cells and thrombocytes in salmonid fish are decreased when levels of stress hormones are experimentally increased (Weinreb 1958; McLeay 1973a, b). Similar alterations in blood cell counts have been reported in fish exposed to a variety of conditions considered as stressful (Weinreb 1958; McLeay 1975).

In a previous study (McLeay 1973c), a 12-h exposure of juvenile coho salmon (*Oncorhynchus kisutch*) to a concentration of kraft pulpmill effluent which was not toxic within 96 h, markedly decreased the white blood cell-thrombocyte counts. This paper describes studies carried out to determine if this blood cell response is indeed a generalized stress response for these fish, and to ascertain if changes in blood cell counts could be used to detect levels of pollutants not acutely toxic to fish.

Materials and Methods

FISH

Coho salmon (*Oncorhynchus kisutch*) fry, obtained as post-alevins from the Washington State Samish Hatchery, were held at low population density in 200-gal fiberglass tanks containing dechlorinated tap water (pH 6.6 ± 0.1, 12 ± 1 C, conductance 5–25 μmho/centimeter, alkalinity 2–8 mg $CaCO_3$/liter, EDTA hardness 3–10 mg $CaCO_3$/liter) for 3–8 mo prior to the studies. They were maintained under natural photoperiod conditions and fed Oregon Moist Pellets 3–5 times daily.

ACUTE STRESS (CROWDING AND ELEVATED TEMPERATURES)

Twelve fish (mean weight, 8.8 ± 2.0 g; fork length, 9.1 ± 0.9 cm) acclimated to 12 ± 1 C were added to each of five polyethylene tanks, 45 × 35 × 40 cm, containing only 2.0 cm depth (2 liters) of dechlorinated water at 20 ± 2 C. Fish loading density was 50 g/liter. The dissolved oxygen content was maintained at saturation levels. Fish were transferred to fresh solutions every 24 h to avoid buildup of metabolic wastes. Groups of 12 fish were killed for blood cell count determinations at 12, 24, 48, 72, and 96 h following transfer from the stock tank. Twelve fish were also sampled directly from the stock tank, just prior to transfer of fish to experimental tanks.

TRANSFER AND ACCLIMATIZATION

Twelve fish (8.5 ± 1.6 g; 8.9 ± 0.8 cm) were placed in 35 liters of dechlorinated water in each of eight Plexiglass compartments, 200 × 12 × 20 cm, painted turquoise blue with numerous black blotches in an attempt to simulate a natural stream environment. Compartments were fitted with translucent lids provided with hinged flaps, to permit feeding of fish without lid removal. Water within each compartment was recirculated at 10 liters/min through a 200-liter reservoir and was renewed daily, providing 2 liters fresh water per gram fish per day. Compartments and reservoirs were housed within a constant temperature (12 ± 1 C) room, free from general laboratory disturbance. Dissolved oxygen content of water within compartments was maintained at saturation by aeration with oil-free compressed air.

Fish in compartments were fed 3 times daily, 8% wet body weight/day; excess food and feces were removed from ends by siphoning. Groups of 12 fish were killed at 12 h and at 1, 2, 4, 7, 14, 21, and 28 days following transfer. Twelve fish were sampled directly from the stock tank, initially and at 28 days.

TIME RESPONSE TO PULPMILL EFFLUENT

Ten fish (2.6 ± 0.9 g; 5.9 ± 0.7 cm) were placed in each of 16 of the stream compartments described previously. Water in each reservoir was renewed daily; fish were fed and ends of the compartments siphoned as before. All fish were acclimatized for 2 wk. Subsequently, 5.25% by volume of neutralized, filtered pulpmill effluent, equivalent to 0.7 of the 96-h LC50 value, was added to reservoirs supplying even-numbered compartments. Odd-numbered compartments served as controls. Effluent was a single sample of untreated whole mill bleached kraft effluent from a B.C. interior mill. This sample was mixed thoroughly and stored in sealed containers at 4 C. Effluent characteristics included: 96-h LC50, 7.5% by volume (as per American Public Health Association 1971, with a fish loading of 0.5 g/liter and solution replacement every 12 h), pH 5.5, 5-day biochemical oxygen demand (BOD_5) 205 mg/liter, total organic carbon (TOC) 245 mg/liter and conductance 970 μmho/centimeter.

During the period of fish exposure to effluents, both effluent or water in the reservoirs were changed every 12 h. Fish were fed three times daily, as previously. Individual groups of control fish and groups of fish exposed to effluent were sacrificed at 1, 4, 8, 12, 24, 48, 72, and 96 h following addition of effluent.

SENSITIVITY TO PULPMILL EFFLUENT

Eight groups of 12 fish (4.7 ± 0.8 g; 7.6 ± 0.4 cm) were acclimatized to individual stream compartments for 2 wk as before. Subsequently, each group was exposed for 24 h to neutralized, filtered pulpmill effluent at concentrations equivalent to 0 (control), 0.04, 0.07, 0.22, 0.37, 0.51, or 0.88 of the effluent's 96-h LC50 value. The effluent was a batch supply of bleached kraft mill effluent from a B.C. coastal mill, with the following characteristics: 96-h LC50, 29% by volume (fish loading 0.5 g/liter, 12-h replacement of test solutions); pH 6.9; BOD_5 187 mg/liter; TOC 245 mg/liter; and conductance 730 μmho/cm.

One group of 12 fish was exposed for 24 h to a portion of this effluent sample treated by foam separation (Ng et al. 1973, 1974) at 20 ± 1 C for 72 h. The concentration of sample examined experimentally was equivalent to 0.51 of the 96-h LC50 value for untreated effluent.

Test solutions of effluent or water in the reservoirs were renewed every 12 h. All groups of fish were

TABLE 1. Effect of acute stress (crowding and elevated temperature) on red blood cell and white blood cell-thrombocyte counts of juvenile coho salmon (*Oncorhynchus kisutch*).

Duration of stress (h)	RBC count ($\times 10^4$ = no./mm^3)	WBC-thrombocyte count ($\times 10^3$ = no./mm^3)
0 (stock)	91.2[a] (16.9)	56.4 (10.9)
12	108.0[b] (17.6)	34.0[d] (14.8)
24	103.5 (21.7)	29.3[d] (4.6)
48	110.1[b] (18.2)	32.1[d] (13.9)
72	95.4 (19.8)	38.0[d] (13.3)
96	94.3 (13.4)	24.1[d] (7.9)

[a]Mean value, with SD in parentheses (as for all Tables).

In Tables 1–5, all P values are based on Student's *t*-test (variances not assumed to be equal), and represent significance of difference between the stock or control sample and the corresponding sample of treated fish. [b]$P < 0.05$, [c]$P < 0.01$, [d]$P < 0.001$.

sacrificed for blood cell counts 24 h after addition of effluent.

Sensitivity to Zinc

Following a 2-wk period of acclimatization to stream compartments, eight groups of 12 fish (7.3 ± 1.1 g; 9.0 ± 0.4 cm) were exposed for 24 h to zinc sulphate at concentrations equivalent to 0 (control), 0.05, 0.1, 0.2, 0.3, 0.7, and 1.2 of the 96-h LC50 value for zinc (1.0 mg Zn/liter determined with 0.5 g fish/liter and 24-h replacement of test solutions). All groups of fish were sacrificed for blood cell counts 24 h after addition of zinc solutions.

Sampling and Analytical Procedures

Individual fish were netted rapidly by random selection; the caudal peduncle was severed and blood collected in a diluting pipette. Red blood cell and white blood cell-thrombocyte counts were determined according to procedures described previously (McLeay 1973c). White blood cells and thrombocytes were counted together since small lymphocytes and immature thrombocytes could not be distinguished in a fresh preparation stained with Rees-Ecker blood-diluting fluid (Klontz and Smith 1968). During the 80–90 min required for sampling each group of 10 or 12 fish, disturbance to the fish was minimized.

Results and Discussion

Response to Acute Stress (Crowding and Elevated Temperature

The effects of acute (up to 96 h) stress, resulting from transferring fish to water at ambient laboratory temperature (20 ± 2 C) and at a depth insufficient to allow free swimming, on numbers of circulating red blood cells and on white blood cell-thrombocyte (WBC-T) counts in juvenile coho salmon are presented in Table 1. Red blood cell counts were slightly increased for two of five exposure periods, while WBC-T counts declined markedly for all exposure times ($P < 0.001$) to 43–67% of the value for the stock fish.

The slight increase in RBC counts within the first 48 h after transfer probably reflects a stress-mediated hemoconcentration due to diuresis, similar to that reported previously (Hunn 1969; Swift and Lloyd 1974).

The marked decrease in WBC-T counts during exposure of fish to stressful conditions for 12–96 h is consistent with the reported decreases in both leukocyte and thrombocyte counts of salmonid fishes exposed to stressful situations and for fish administered ACTH or corticosteroids (Weinreb 1958; McLeay 1973a, 1973b, 1975). Circulating white blood cells in immature coho salmon are predominantly lymphocytes, which in vertebrates are susceptible to lysis by corticosteroids (Dougherty 1960; Bennett and Harbottle 1968; Bennett et al. 1972). Lymphopenia due to stress has been shown in other lower vertebrates including goldfish (Bennett and Neville 1975) and newts (Bennett and Johnson 1973). This association of a lymphopenic response with the stress response is well known in mammals (Dalton and Selye

TABLE 2. Effect of transfer and acclimatization to experimental streams on red blood cell and white blood cell-thrombocyte counts of juvenile coho salmon.

Time after transfer (*days*)	RBC count ($\times 10^4$ = *no./mm³*)	WBC-thrombocyte count ($\times 10^3$ = *no./mm³*)
0 (stock)	105.2	51.2
	(12.1)	(11.5)
0.5	101.0	36.3[c]
	(16.7)	(9.5)
1	111.1	34.1[d]
	(19.0)	(4.6)
2	106.8	45.4
	(13.5)	(15.2)
4	114.7	52.1
	(17.3)	(9.5)
7	119.1[c]	73.3[d]
	(12.1)	(14.0)
14	106.2	88.0[d]
	(14.0)	(19.4)
21	106.1	82.2[c]
	(16.8)	(21.2)
28	100.0	78.4[d]
	(12.9)	(16.5)
28-day stock	91.2[b]	56.1
	(17.3)	(11.0)

All *P* values represent significance of difference between the initial stock sample and each transferred sample. (See Table 1 footnotes.)

1938), and reflects the alarm reaction of the general adaption syndrome to nonspecific stress (Selye 1950). The depression of the WBC-T count in fish after 12 h in the present study showed no signs of recovery within 96 h; therefore, this response is maintained throughout the acute (4-day) period provided the stressful stimulus is continued.

RESPONSE TO TRANSFER TO STREAM AQUARIA

Transfer of fish from the stock tank to stream compartments containing fresh water did not alter RBC counts over the following 28 days except for an increase at 7 days (Table 2). In contrast, WBC-T counts were below stock values ($P < 0.01$) at 12 and 24 h, returning to levels of stock fish by 2–4 days and increasing ($P < 0.01$) above values for the stock fish at 7–28 days.

The initial decrease in WBC-T counts is interpreted as an acute stress response due to transfer. Transferring goldfish between identical aquaria (Chavin and Young 1970) or juvenile coho salmon from a stock tank to aquaria (McLeay and Brown 1975) resulted in a rapid rise in blood sugar levels which was maintained for up to 4 days. Like the WBC-T response, hyperglycemia is evoked in fish also by administration of the stress hormones ACTH (Oguri and Nace 1966) and cortisol (Robertson et al. 1963), and by catecholamines (Young and Chavin 1965). External stressors elicit rapid increases in the levels of these hormones within the circulatory system (Nakano and Tomlinson 1967; Wedemeyer 1969; Chavin 1973).

The increase in the WBC-T counts relative to stock values at 7–28 days following transfer is considered to indicate that this environment was less stressful to the fish than that in the stock tank. This response is not considered to be a typical over-compensation response to a prolonged stressful situation or to the initial stress of transfer. Previous studies (McLeay 1973c; McLeay and Brown 1974) have shown that WBC counts of juvenile coho salmon taken after 25 or 200 days continuous exposure to pulpmill effluent were the same as in control fish.

TABLE 3. Effect with time of 0.7 LC50 BKME[a] on red blood cell and white blood cell-thrombocyte counts of juvenile coho salmon.

Exposure period (*h*)	RBC count ($\times 10^4$ = *no./mm³*)		WBC-thrombocyte count ($\times 10^3$ = *no./mm³*)	
	Control (*water*)	Effluent	Control (*water*)	Effluent
1	112.8 (10.9)	98.4 (21.1)	56.9 (14.8)	56.1 (10.5)
4	103.2 (19.8)	116.3 (20.0)	55.8 (19.7)	54.0 (11.1)
8	106.0 (15.0)	103.4 (9.0)	63.8 (10.5)	49.8[c] (9.2)
12	103.7 (26.8)	105.4 (19.1)	63.0 (18.3)	55.3 (11.4)
24	110.6 (14.7)	96.0[b] (16.1)	63.1 (14.0)	43.6[d] (8.1)
48	107.2 (14.0)	97.9 (10.3)	63.9 (12.6)	49.5[c] (11.2)
72	105.1 (16.1)	95.4 (16.2)	61.2 (8.1)	54.9 (17.5)
96	102.3 (12.4)	87.5 (12.3)	58.6 (10.0)	42.6[c] (18.4)

[a]Neutralized, filtered bleached kraft mill effluent concentration expressed as a fraction of its 96-h LC50 value.
[b,c,d]Table 1.

TIME RESPONSE TO PULPMILL EFFLUENT

The effects of the duration of exposure of acclimatized fish to pulpmill effluent (concentration 0.7 of the sample's 96-h LC50 value) for 1–96 h on their blood cell counts is shown in Table 3. RBC counts were similar for all control samples. Mean values for RBC counts in effluent-exposed fish were decreased from corresponding control values at 24–96 h; however, differences were only significant ($P < 0.05$) at 24 h. In all control samples WBC-T counts were similar; values were decreased ($P < 0.01$) in effluent-exposed fish at 8, 24, 48, and 96 h.

In an earlier study (McLeay 1973c) WBC-T counts were decreased by 50% and RBC counts unchanged in juvenile coho salmon exposed to pulpmill effluent for 12 h following a 2-wk acclimatization period; WBC-T counts were indistinguishable from control values when exposures were extended to 25 days. After short-term exposure, numbers of circulating small lymphocytes were decreased, whereas numbers of thrombocytes and other leukocyte cell types making up the WBC-T count were not decreased significantly. These and the present data reflect a pattern of response similar to that of circulating lymphocytes in mammals, both during the alarm reaction to nonspecific stress (lymphopenia) and during the subsequent stage of resistance to stress (recovery), as described by Selye (1950, 1971).

SENSITIVITY TO PULPMILL EFFLUENT

The response of WBC-T counts in acclimatized fish exposed for 24 h to a range of sublethal (i.e. less than the 96-h LC50) concentrations of pulpmill effluent was examined since sensitivity of the response was greatest for this exposure period. As shown in Table 4, RBC counts were unchanged for all effluent concentrations except the highest. However, the WBC-T counts were decreased ($P < 0.001$) in fish exposed to untreated effluent concentrations of 0.2 LC50 and higher. Foam fractionation of the effluent diminished the response considerably. At a concentration equivalent by volume to 0.5 of the 96-h LC50 value, the response for treated effluent was barely significant ($P < 0.05$) whereas for untreated effluent the response was marked ($P < 0.001$).

The slight decrease in RBC counts in fish after 24-h exposure to the highest concentration of pulpmill effluent was observed previously (Table 3) with a sample of effluent from another mill.

TABLE 4. Effect of 24-h exposure to varying BKME concentrations on red blood cell and white blood cell-thrombocyte counts of juvenile coho salmon.

BKME[a] concentration (*as fraction of its 96-h LC50 value*)	RBC count ($\times 10^4$ = *no./mm³*)	WBC-thrombocyte count ($\times 10^3$ = *no./mm³*)
0 (control)	121.1 (17.0)	73.1 (13.1)
0.04	118.3 (19.2)	71.3 (13.8)
0.07	111.0 (13.9)	65.2 (15.6)
0.22	112.3 (15.1)	46.2[d] (11.4)
0.37	118.2 (22.7)	45.1[d] (12.0)
0.51	115.4 (14.0)	45.1[d] (10.7)
0.51 treated[c]	111.2 (18.1)	62.4[b] (8.5)
0.88	106.1[b] (13.4)	32.7[d] (11.8)

[a]Neutralized, filtered bleached kraft mill effluent.
[c]Foam-stripped effluent; concentration equivalent by volume to 0.51 of the 96-h LC50 value for untreated effluent.

TABLE 5. Effect of 24-h exposure to varying zinc concentrations on red blood cell and white blood cell-thrombocyte counts of juvenile coho salmon.

Zinc concentration (*as fraction of its 96-h LC50 value*)	RBC count ($\times 10^4$ = *no./mm³*)	WBC-thrombocyte count ($\times 10^3$ = *no./mm³*)
0 (control)	112.1 (26.9)	89.2 (27.5)
0.05	110.8 (24.7)	87.0 (12.6)
0.10	102.0 (15.1)	75.5 (15.7)
0.20	103.9 (19.0)	76.0 (23.7)
0.30	108.7 (16.6)	72.6 (27.1)
0.50	103.2 (13.0)	67.0[c] (12.5)
0.70	104.9 (20.1)	45.5[d] (9.8)
1.20	98.6 (16.6)	39.1[d] (10.9)

This observation and the reductions in hematocrit values of fish exposed to pulpmill effluents observed by other investigators (Howard and Walden 1967; Warner 1967) may be manifestations of the same phenomenon.

Since the WBC-T count in fish exposed for only 24 h to pulpmill effluent was depressed significantly at concentrations as low as 0.2 of the sample's 96-h LC50 concentration, this response is a sensitive index of the threshold levels of pulpmill effluent which are stressful to fish. The technique also indicated an effect attributable to a 15% concentration of a treated (foam-stripped) effluent which was not lethal to fish at 100% concentration; therefore, this procedure may be used to monitor the efficiency of waste treatment in removing sublethal levels of effluent toxicity.

Sensitivity to Zinc

The WBC-T count response was used to examine the sublethal toxicity of zinc in order to determine the usefulness of the technique for pollutants other than pulpmill effluents. The RBC counts in fish exposed to zinc for 24 h at concentrations up to 1.2 of the 96-h LC50 value were unchanged (Table 5). Mean WBC-T counts were decreased at all concentrations, although differences were only significant ($P < 0.01$) at values of 0.5 LC50 and greater.

This lack of sensitivity of the WBC-T response to acutely sublethal concentrations of zinc means that the procedure developed for pupmill effluents is not directly applicable to zinc. The lack of response may be because experimental conditions, such as exposure time, did not correspond to the optimum period for a maximum response to this toxicant. Additional studies are necessary to determine if the WBC-T count technique is a valid indicator of the sublethal toxicity of pollutants other than pulpmill effluents.

Conclusions

These experiments have demonstrated that the WBC-T count (no./mm^3 blood) in salmon is extremely sensitive to acute stress. Mere transfer of fish from stock tanks depressed WBC-T counts, requiring 2–4 days for values to recover. The RBC count is far less sensitive to stress.

Measurement of the WBC-T count response in fish appears to provide a reasonably rapid, sensitive, and quantitative method for determining stressful levels of pulpmill effluents to fish. Development of this or some sublethal response into a routine bioassay test is essential for monitoring safe discharge limits of pollutants to receiving waters, particularly in many mill-treated pulpmill wastes, where volumes are exceptionally large, but the discharge is not acutely lethal. The major disadvantage of the WBC-T response as a sublethal test procedure is its sensitivity to nonspecific stressors, thus requiring acclimatization of fish and carefully-controlled experimental conditions.

Depression of the leukocyte–thrombocyte counts in salmon due to stress is attributable to a decrease in the number of circulating small lymphocytes (McLeay 1973c). At least in mammals, this cell type is intimately involved in the production of antibodies (Roberts 1960; Nossal and Ada 1971) which are a major factor in disease resistance. It is suggested that the well-known decreased disease resistance of fish due to stress (Wedemeyer 1970; Snieszko 1974) is related to a stress-induced lymphopenia.

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Estimating Survival Rate from Tag Returns: Model Tests and Sample Size Determination

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This paper brings to the attention of fishery biologists a method of estimating survival and exploitation rates when a series of tag recaptures from angler-killed fish is available; this model is appropriate only for recaptures that are removed from the population. Tests for the appropriateness of the model and methods for determining sample size are presented. The test for the model is imperative since estimates of survival would be meaningless if data do not conform to model.

YOUNGS, W. D., AND D. S. ROBSON. 1975. Estimating survival rate from tag returns: model tests and sample size determination. J. Fish. Res. Board Can. 32: 2365–2371.

Cet article veut porter à l'attention des biologistes des pêches une méthode d'estimation des taux de survivance et d'exploitation à partir d'une série de retours de marques de poissons capturés à la ligne; ce modèle ne vaut que si les poissons recapturés sont enlevés de la population. Nous décrivons les tests permettant de vérifier la pertinence du modèle et les méthodes selon lesquelles on peut déterminer la grosseur de l'échantillon. L'épreuve du modèle est essentielle, car les estimations de survivance seraient sans valeur si les données ne se conformaient pas au modèle.

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SUCCESSFUL management of a fishery nearly always requires information on survival and exploitation rates. Numerous methods have been developed for estimating survival rates depending upon certain basic assumptions, i.e. survival is constant or variable. Methods for estimating exploitation rates are not as well developed and most are costly and imprecise.

The model proposed in this paper is based upon the assumption of year-specific, age-independent survival, and reported exploitation rates, and is based on angler return of tags. Unless year-class cohorts are recorded and analyzed separately, this model requires equal vulnerability to angling of all tagged fish and between tagged and untagged fish. A stochastic model for mortality and reporting is also assumed; the statistical development of this model has been treated independently by Seber (1970) and Robson and Youngs (1971).

A Recapture Model

A sample of fish is annually taken from the population, tagged with numbered tags, and returned (any live recaptures of previously tagged fish are ignored in this analysis of angler tag recoveries). Tags normally bear a printed request for the angler to return the tag, stating the date, area, and size of the fish when captured. After a period of k years a body of recapture information is available from angler-reported tag recoveries and could be represented by Table 1.

A tagged fish alive at the start of a year may suffer any one of three fates during the course of the year: 1) may be harvested during the year, 2) may be otherwise killed during the year, or 3) may survive the year. These are conventionally represented by the following symbols:

E_i = Probability of being harvested during year i
S_i = Probability of surviving year i
$1 - S_i - E_i$ = Probability of being killed otherwise during year i

An angler-harvested tag may go unreported, and it is assumed only that the probability of reporting a tag, given a tagged fish has been caught, is independent of the year of tagging. This conditional probability is designated λ_i.

If each tagged fish, when mixed with the larger

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TABLE 1. Array of angler reported tag recoveries over a period of k years.

Year tagged	Number tagged	Reported recaptured in year 1	2	3	...	k	Total recaptures
1	N_1	R_{11}	R_{12}	R_{13}	...	R_{1k}	R_1
2	N_2		R_{22}	R_{23}	...	R_{2k}	R_2
3	N_3			R_{33}	...	R_{3k}	R_3
.	.			.		.	.
.	.				.	.	.
.	.					.	.
k	N_k					R_{kk}	R_k
Year total recaptures		C_1	C_2	C_3	...	C_k	

exploited population, suffers a statistically independent fate, a likelihood function for the entire recapture array may be expressed as the product of multinomial distributions. For fixed numbers of tag releases $N_1, N_2, \ldots, N_k$, and letting $\{R_{ij}\}$ stand for the array of recaptures in Table 1, the likelihood function becomes

$$P(\{R_{ij}\}) = \prod_{i=1}^{k} \binom{N_i}{R_{ii}, \ldots, R_{ik}, N_i - R_i} \times (\lambda_i E_i)^{R_{ii}} (\lambda_{i+1} E_{i+1} S_i)^{R_{i,i+1}} \ldots (\lambda_k E_k S_i \ldots S_{k-1})^{R_{ik}} (1 - \lambda_i E_i - \lambda_{i+1} E_{i+1} S_i - \ldots - \lambda_k E_k S_i S_{i+1} \ldots S_{k-1})^{N_i - R_i}$$

Robson and Youngs (1971) showed that the summary array of row and column totals, R_i and C_i, respectively, from Table 1 constitute a minimal sufficient statistic, i.e. R_i and C_i contain all the information present for estimation of survival and exploitation rates. Defining $T_1 = R_1$, and $T_{i+1} = R_{i+1} + T_i - C_i$ for $i = 1$ to $k - 1$, so that T_i is the number of tag recoveries after the i'th release from fish tagged on or before the i'th release, we may reparameterize the likelihood function as

$$P(\{R_{ij}\}) = \prod_{i=1}^{k} \binom{N_i}{R_{ii} \ldots R_{ik}, N_i - R_i} E_i^{*C_i} S_{i-1}^{T_i - R_i} (1 - \rho_i)^{N_i - R_i}$$

where E_i^* is the reported exploitation rate, $E_i^* = \lambda_i E_i$, and ρ_i is the probability that a tagged fish released at the start of year i will be harvested and reported before the experiment terminates,

$$\rho_i = E_i^* + E^*_{i+1} S_i + \ldots + E_k^* S_i S_{i+1} \ldots S_{k-1}.$$

Maximum likelihood estimators of E_i^* and S_i in terms of C_i, T_i, and $r_i = \frac{R_i}{N_i}$ are

$$\hat{E}_i^* = r_i \frac{C_i}{T_i} \quad \text{and} \quad \hat{S}_i = \frac{r_i - \hat{E}_i^*}{r_{i+1}}$$

These estimators are consistent for large N_i, and E_i^* is an unbiased estimator of E_i^*; the bias of S_i is approximately equal to

$$-\hat{S}_i (1 - \rho_{i+1})^{N_{i+1}}$$

With no further assumptions the factors λ_i and E_i in $E_i^* = \lambda_i E_i$ are inseparable, and Youngs (1974) has given some necessary conditions for estimating E from E*. Variances and covariances for these estimators are approximately given by the following:

$$\hat{\sigma}_{E_i^*}^2 \doteq \frac{E_i^*}{R_i N_i} \times \left[\hat{E}_i^* (N_i - R_i) + \frac{R_i (R_i - \hat{E}_i^* N_i)}{T_i} \right]$$

$$\hat{\sigma}_{\hat{S}_i}^2 \doteq \hat{S}_i^2 \left[\frac{N_i - R_i}{N_i R_i} + \frac{N_{i+1} - R_{i+1}}{N_{i+1} R_{i+1}} + \frac{\hat{E}_i^* N_i}{T_i (R_i - N_i \hat{E}_i^*)} \right]$$

$$\hat{\sigma}_{\hat{E}_i^* \hat{S}_i} \doteq \hat{E}_i^* \hat{S}_i \left[\frac{N_i - R_i}{N_i R_i} - \frac{1}{T_i} \right]$$

$$\hat{\sigma}_{\hat{E}_i^* \hat{E}_{i+j}^*} \doteq 0$$

$$\hat{\sigma}_{\hat{E}_i^* \hat{S}_{i+j}} \doteq 0$$

$$\hat{\sigma}_{\hat{E}_i^* \hat{S}_{i+j}} \doteq \begin{cases} 0 & \text{for } j > 1 \\ -\dfrac{\hat{E}^*_{i+1} \hat{S}_i}{N_{i+1} R_{i+1}} (N_{i+1} - R_{i+1}) & \text{for } j = 1 \end{cases}$$

$$\hat{\sigma}_{\hat{S}_i\hat{S}_{i+j}} \doteq \begin{cases} 0 & \text{for } j > 1 \\ -\dfrac{\hat{S}_i\hat{S}_{i+1}}{N_{i+1}R_{i+1}}(N_{i+1} - R_{i+1}) & \text{for } j = 1 \end{cases}$$

Planning Studies

It is possible with approximate estimates of survival and reported exploitation rates to determine the number of tagged fish to be released in order to obtain estimates with desired accuracy. This information can serve as a guide in planning studies when survival and exploitation are to be estimated. Variances of these estimates are functions of the survival and exploitation rates, the number of years involved, and the number of fish tagged.

Graphs may be developed to facilitate judgment as to the number of fish to tag and length of time to carry out the study. Each graph has the ordinate equal to the standard deviation of the estimator times the square root of number tagged. The abscissal value is for the parameter under consideration, i.e. either survival or exploitation. A separate graph could be constructed for $\sigma_{\hat{S}_1}\sqrt{N}$ as a function of number of years tagged (k), S, and E*. Examples for $\sigma_{\hat{S}_1}\sqrt{N}$ for E* equal to 0.01, 0.1, and 0.3 are shown in Fig. 1–3, respectively.

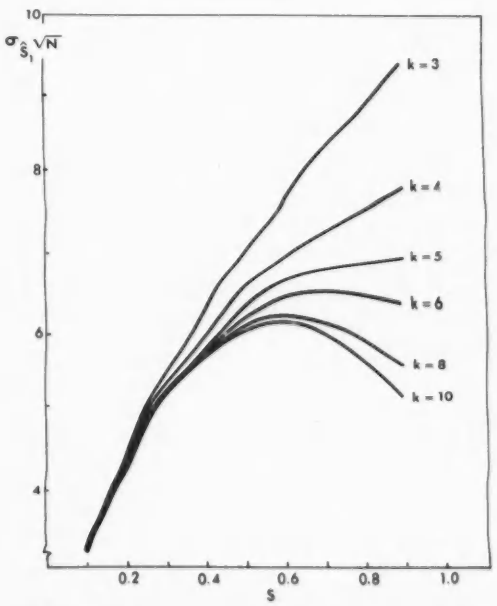

FIG. 1. Relation of $\sigma_{\hat{S}_1}\sqrt{N}$ as a function of survival (S) and number of years of tag releases (k) with a reported exploitation rate (E*) equal to 0.01.

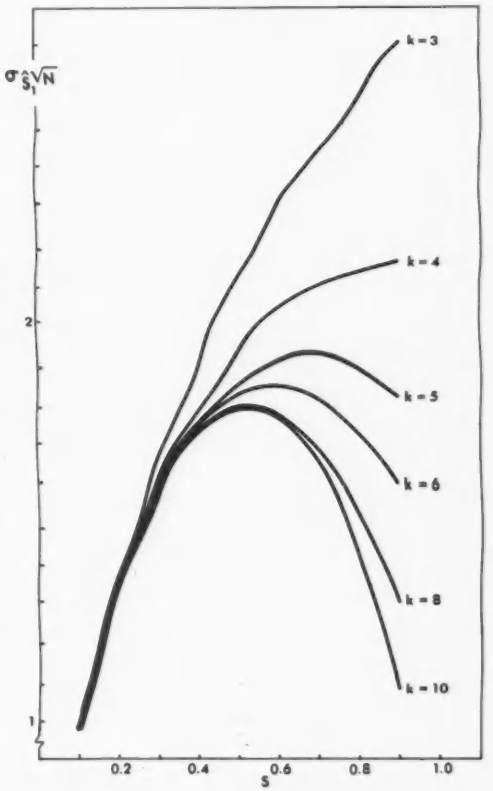

FIG. 2. Relation of $\sigma_{\hat{S}_1}\sqrt{N}$ as a function of survival (S) and number of years of tag releases (k) with a reported exploitation rate (E*) equal to 0.1.

The use of such charts may be illustrated by a specific example. Suppose a fishery manager wishes to estimate survival during the first year of study for a particular population within ± 0.1 with a 1 in 20 chance of being wrong on the average, or formulated as a confidence statement

$$P(\hat{S} - 0.1 \leqslant S \leqslant \hat{S} + 0.1) = 0.95$$

Suppose further that preliminary studies have indicated a survival of approximately 0.6 with reported exploitation of approximately 0.1. From the above confidence statement

$$\sigma_{\hat{S}_1} = \frac{0.1}{t_{0.95}} \approx \frac{0.1}{2} = 0.05$$

Therefore, $\sigma_{\hat{S}_1}$ must be near 0.5 to have the desired level of confidence. Since E_1* is approximately 0.1, the graph in Fig. 2 is appropriate.

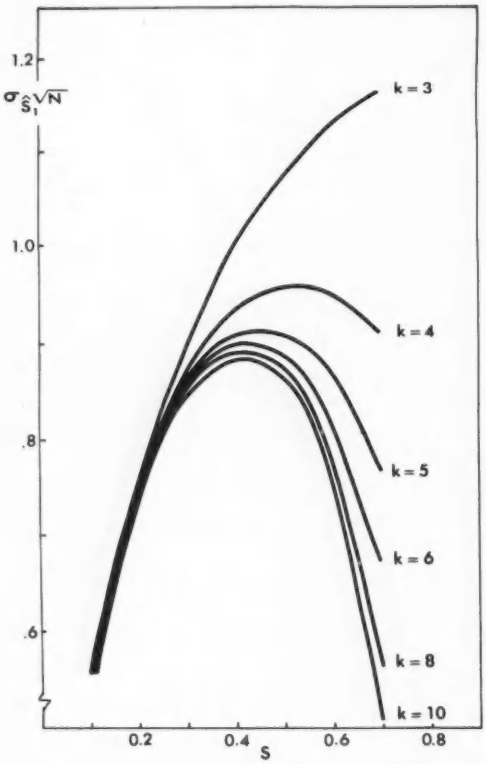

FIG. 3. Relation of $\sigma_{\hat{S}_1}\sqrt{N}$ as a function of survival (S) and number of years of tag releases (k) with a reported exploitation rate (E*) equal to 0.3.

This graph gives values of $\sigma_{\hat{S}_1}\sqrt{N}$, at S equal 0.6, ranging from 1.78 for $k = 10$ years up to 2.30 for $k = 3$ yr. If the study were to be carried out for only 3 yr, the number of tags released each year would have to be equal to

$$N = \left[\frac{2.30}{0.05}\right]^2 = 2116$$

A study period carried out for $k = 10$ yr would require

$$N = \left[\frac{1.78}{0.05}\right]^2 = 1267$$

The values used to generate these graphs may be computed from the following

$$\sigma_{\hat{S}_i}\sqrt{N} = \left\{\frac{S^2}{E^*}\left[\frac{1 - S^2}{S(1 - S^{k-i})} - \frac{(1 - S)^2(1 - S^{i-1})}{(1 - S^i)(1 - S^{k-i})(1 - S^{k-i+1})} - 2E^*\right]\right\}^{1/2}$$

where i refers to the year in question and k is the number of years of tagging and recapture.

The values of $\sigma_{\hat{S}_1}\sqrt{N}$ for any given i decrease with increasing E* but, for increasing 1, the values of $\sigma_{\hat{S}_1}\sqrt{N}$ have variable response depending upon k, i, and E*. These responses are shown qualitatively in Fig. 4 for $k = 6$ and E* = 0.1 and 0.3. This general response of lower values of $\sigma_{\hat{S}_1}\sqrt{N}$ for midrange values of i is true for all $k > 3$; with $k = 3$ there are only two i's ($i = 1$ and 2) and the value of $\sigma_{\hat{S}_2}\sqrt{N}$ is greater than $\hat{\sigma}_{S_1}\sqrt{N}$. The relationships between $\sigma_{\hat{S}_1}\sqrt{N}$ and S are functionally like curves in Fig. 1–3 in which for $k > 5$, $k > 4$, $k > 3$, respectively, there is a maximum of $\sigma_{\hat{S}_1}\sqrt{N}$ for intermediate values of S. The maximum value of $\sigma_{\hat{S}_1}\sqrt{N}$ is a function of E* and occurs when $i = 2$ and $k = 3$ with S at its maximum possible value. This is shown graphically in Fig. 5 and serves to place an upper bound on the number of tags released each year in order to have at least the desired level of confidence.

The minimum value of $\sigma_{\hat{S}_1}\sqrt{N}$ occurs at maximum E* and is only weakly dependent on k and i. This is apparent from inspection of Fig. 1–3 observing the convergence of lines as S decreases. The values of $\sigma_{\hat{S}_1}\sqrt{N}$ range between 0.285 and 0.305 with E* = 0.9, $k = 3, 4, \ldots, 10$ and $i = 1, 2, \ldots, k - 1$.

Values for $\sigma_{\hat{E}_i^*}\sqrt{N}$ may be dealt with in similar fashion with graphs generated from

$$\sigma_{\hat{E}_i^*}\sqrt{N} = \left\{\frac{E^*}{1 - S^{k-i+1}}\left[1 - S - E^*(1 - S^{k-i+1}) + S(1 - S)\frac{1 - S^{k-i}}{1 - S^i}\right]\right\}^{1/2}$$

The variance of $\hat{E}_i^*$ is less dependent on other parameters of the model, is smaller than the variance of S_i, decreases as S increases, and behaves in a binomial fashion with maximum values occurring at intermediate values of E* for any given S.

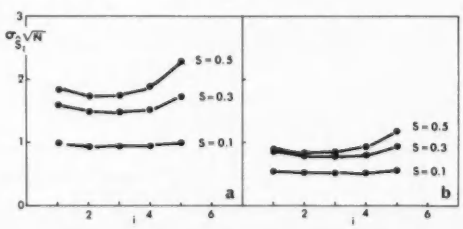

FIG. 4. Response of $\sigma_{\hat{S}_i}\sqrt{N}$ as a function of i for $k = 6$, and E* = 0.1 (a) and 0.3 (b).

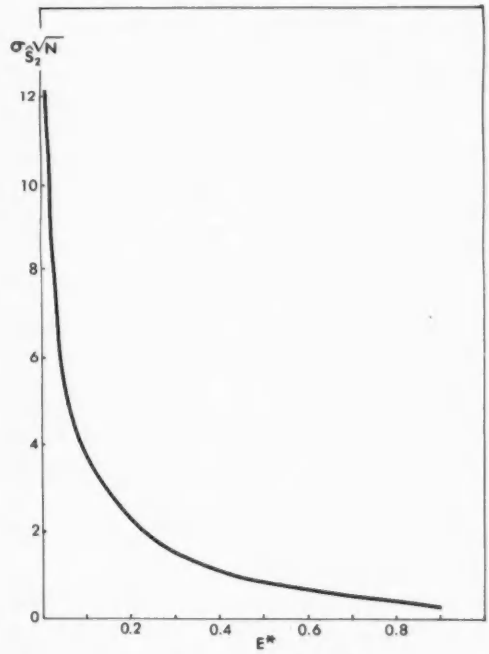

FIG. 5. Relationship between $\sigma_{\hat{S}_2}\sqrt{N}$ and E* for $k = 3$ yr of tag releases.

Testing the Model

A contingency chi-square test can be applied to the $2 \times (k-1)$ table:

Year tagged	Yr of recapture: 2	3	...	k	Total
1	R_{12}	R_{13}	...	R_{1k}	$T_2 - R_2$
2	R_{22}	R_{23}	...	R_{2k}	R_2
Total	C_2	R_{23}^*	...	R_{2k}^*	T_2

Another contingency chi-square test of the $2 \times (k-2)$ table:

Year tagged	Yr of recapture: 3	4	...	k	Total
1 or 2	R_{23}^*	R_{24}^*	...	R_{2k}^*	$T_3 - R_3$
3	R_{33}	R_{34}	...	R_{3k}	R_3
Total	C_3	R_{34}^*	...	R_{3k}^*	T_3

may be constructed which is independent (asymptotically) of the preceding test, and the two chi-square statistics are additive. This argument continues in the obvious manner resulting in $k-2$ independent contingency chi-square statistics which may be added to give one combined test of the model with $(k-1)(k-2)/2$ df (degrees of freedom). If marginal totals are too small for validity of the chi-square approximation, grouping will be necessary with a corresponding reduction in degrees of freedom.

Certain alternatives to the model may be tested by partitioning chi-square. An alternative that is likely to occur in a fish-tagging experiment is a differential mortality rate and/or vulnerability to anglers immediately after release of the tagged fish, followed by a return to normal before the next anniversary date. Tag returns in this case would be expected to follow the modified pattern in Table 2.

A test against this alternative is obtained by partitioning each $2 \times (k-i+1)$ table:

$R_{i-1,i}^*$	$R_{i-1,i+1}^*$	....	$R_{i-1,k}^*$	$T_i - R_i$
$R_{i,i}$	$R_{i,i+1}$	....	$R_{i,k}$	R_i
C_i	$R_{i,i+1}^*$	....	$R_{i,k}^*$	T_i

$\rightarrow \chi^2_{k-i}$

into two tables:

$R_{i-1,i}^*$	$T_i - R_i - R_{i-1,i}^*$	$T_i - R_i$
$R_{i,i}$	$R_i - R_{ii}$	R_i
C_i	$T_i - C_i$	

$\rightarrow \chi_1^2$

and

$R_{i-1,i+1}$	$R_{i-1,k}$	$T_i - R_i - R_{i-1,i}^*$
$R_{i,i+1}$	$R_{i,k}$	$R_i - R_{ii}$
R_{ii}	$R_{i,k}^*$	$T_i - C_i$

$\rightarrow \chi^2_{k-i-1}$

TABLE 2. Expected tag returns if differential rates occur during the first year after release.

Tag releases: Year	Number	Year of return: 1	2	3	....	k
1	N_1	$N_1E_1^{**}$	$N_1S_1^*E_2^*$	$N_1E_3^*S_1^*S_2$	....	$N_1E_k^*S_1^*S_2 \ldots S_{k-1}$
2	N_2		$N_2E_2^{**}$	$N_2E_3^*S_2^*$	....	$N_2E_k^*S_2^*S_3 \ldots S_{k-1}$
3	N_3			$N_3E_3^{**}$	....	$N_3E_k^*S_3^* \ldots S_{k-1}$
.	.				.	.
.	.				.	.
k	N_k					$N_kE_k^{**}$

TABLE 3. Array of angler reported tag recoveries of tagged lake trout, Cayuga Lake (Youngs, 1972).

Year tagged (i)	Number tagged (N_i)	Recaptured in year										Total (R_i)
		60–61	61–62	62–63	63–64	64–65	65–66	66–67	67–68	68–69	69–70	
1960	1048	72	44	8	9	4	4	1	1	1		144
1961	844		74	30	20	7	4	2	1			138
1962	989			54	48	13	23	5	4	2		149
1963	971				74	24	16	7	3	1	1	126
1964	863					48	40	5	5	2	5	105
1965	465						31	10	6	3	2	52
1966	845							38	30	6	2	76
1967	360								19	6	6	31
1968	625									13	14	27
1969	760										21	21
Total (C_i)		72	118	92	151	96	118	68	69	34	51	
T_i		144	210	241	275	229	185	143	106	64	51	
$\hat{S}_i$		0.420	0.476	0.718	0.483	0.632	0.450	0.550	0.696	0.733		
$\pm 1.96\sigma_{S_i}$		±0.116	±0.125	±0.173	±0.133	±0.210	±0.173	±0.235	±0.392	±0.453		
$\hat{E}_i^*$		0.0687	0.0919	0.0575	0.0710	0.0510	0.0713	0.0427	0.0561	0.0230	0.0276	
$\pm 1.96\sigma_{\hat{E}_i}$		±0.0153	±0.0178	±0.0126	±0.0139	±0.0120	±0.0198	±0.0118	±0.0204	±0.0100	±0.0116	

TABLE 4. Contingency chi-square tests of goodness of fit to the tag recapture model.

Year tagged	Year of recapture									Two row contingency chi-square	First column vs. remaining chi-square
	61–62	62–63	63–64	64–65	65–66	66–67	67–68	68–69	69–70		
1960	44	8	9	4	4	1	1	1	0		
										5.185	0.279
1961	74	30	20	7	4	2	1	0	0	(5 df)	(1 df)
1960–61	118	38	29	11	8	3	2	1	0		
										3.086	3.390
1962		54	48	13	23	5	4	2	0	(5 df)	(1 df)
1960–62		92	77	24	31	8	6	3	0		
										4.219	0.544
1963			74	24	16	7	3	1	1	(5 df)	(1 df)
1960–63			151	48	47	15	9	4	1		
										7.268	1.146
1964				48	40	5	5	2	5	(5 df)	(1 df)
1960–64				96	87	20	14	6	6		
										0.798	1.371
1965					31	10	6	3	2	(4 df)	(1 df)
1960–65					118	30	20	9	8		
										6.601	0.048
1966						38	30	6	2	(3 df)	(1 df)
1960–66						68	50	15	10		
										0.629	1.078
1967							19	6	6	(2 df)	(1 df)
1960–67							69	21	16		
										0.465	0.465
1968								13	14	(1 df)	(1 df)
									Total	28.251	5.321
										30 df	8 df

The sum of these $k - 2$ single degrees of freedom chi-squares provides a test against the short-term differential effects of tagging. Continued partitioning in this manner clearly provides tests against successively longer term perturbations following tagging. Year-specific survival rates are not estimable from angler tag return data which the model in Table 2 obtains but do become estimable from live recapture data (Robson 1969), so this chi-square test is critically important to the validity of the killed recapture method.

An Example

Youngs (1972) presented data from a 10-yr tagging experiment on lake trout in Cayuga Lake. These data are presented in Table 3 along with estimates of S_i and E_i* with respective 95% confidence intervals.

Calculations for chi-square goodness of fit tests are given in Table 4. Grouping was necessary for some years with limited recaptures resulting in a decrease of degrees of freedom. For example, when testing 1960–61 vs. 1962 tag recapture data, year-of-recapture data from 1966 to 1967 on is pooled providing a χ^2 value of 3.086 with 5 df. None of the chi-square values was critical; data and model were judged in good agreement. Likewise, the pooled chi-square testing first-year differences was not significant, leading to the conclusion of no differential loss or vulnerability of tagged fish during the year immediately following release.

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Penetration of the Deep Layer of the Saguenay Fjord by Surface Waters of the St. Lawrence Estuary[1]

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THERRIAULT, J. C., AND G. LACROIX. 1975. Penetration of the deep layer of the Saguenay fjord by surface waters of the St. Lawrence estuary. J. Fish. Res. Board Can. 32: 2373–2377.

A strong similarity is demonstrated in summertime physicochemical characteristics between the deep water of the Saguenay fjord and the surface water of the St. Lawrence estuary. Summer warming of the deep layer of the Saguenay is progressive from the mouth towards the head of the fjord. The mechanism proposed is the penetration of surface estuarine water over the shallow sill during the rising tide. The abnormally high chlorophyll values in this deep layer may be explained by the same advective mechanism.

THERRIAULT, J. C., AND G. LACROIX. 1975. Penetration of the deep layer of the Saguenay fjord by surface waters of the St. Lawrence estuary. J. Fish. Res. Board Can. 32: 2373–2377.

Une série saisonnière de mesures faites pendant l'été dans le Saguenay et dans les eaux adjacentes de l'estuaire du Saint-Laurent a montré la similitude physique et chimique (oxygène, sels nutritifs) des eaux profondes du Saguenay et des eaux de surface de l'estuaire du Saint-Laurent. Elles ont permis de déterminer que le réchauffement estival des eaux profondes du Saguenay se fait progressivement de l'aval vers l'amont et qu'il est relié à la pénétration, au-dessus du seuil d'eaux de surface de l'Estuaire pendant la marée montante. Les concentrations anormalement élevées de chlorophylle des eaux profondes de l'aval du Saguenay s'expliquent également par ce mécanisme advectif.

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GIVEN the magnitude of its flow (annual mean about 1.3×10^3 m^3/s (d'Anglejan 1970), and the unique geographical location of its outflow (Fig. 1), the Saguenay fjord is undoubtedly the most important tributary of the St. Lawrence estuary. It flows 164 km from its source in Lac St. Jean (48°35′ N lat. and 71°45′ W long.) before discharging into the St. Lawrence estuary only a few kilometers from the Laurentian Channel. For the last 100 km the flow is via a deep fjord (up to 275 m) with a sill of only 20-m depth (Fig. 2). Vertical tidal displacement in the Saguenay is from 3 to 6 m (Canadian Hydrographic Service 1971). At the mouth of the Saguenay, the current varies between 1 and 2 m S^{-1} during the flood and between 1.5 and 2.5 during the ebb (Service Hydrographique du Canada 1973).

Drainville (1968) has characterized the stratification of the Saguenay as two distinct layers separated by a strong thermohalocline: 1) a thin superficial layer (0–25 m) consisting of fairly warm water of low salinity with vertical gradients very pronounced; 2) a much more uniform, deeper layer characterized by cold, saline water, well oxygenated down to the bottom. To account for the characteristics of the deep layer, Drainville (1968) suggested two hypotheses: 1) penetration of surface water from the St. Lawrence estuary over the sill in compensation for the outflow from the Saguenay and 2) total vertical mixing in winter of the waters of the Saguenay.

In this paper we examine in detail the first of these hypotheses in the light of data on temperature and salinity as well as dissolved oxygen, phosphate, nitrate, and chlorophyll, and their seasonal variations.

Material and Methods

Water samples were taken at three stations in the Saguenay during the summer of 1971 (Fig. 1). These were Station DR 440 situated at the mouth, just inside the sill (Fig. 2), and Stations DR 400A and DR 330, situated about 20 and 40 km, respectively, inside the fjord. In the St. Lawrence estuary at the

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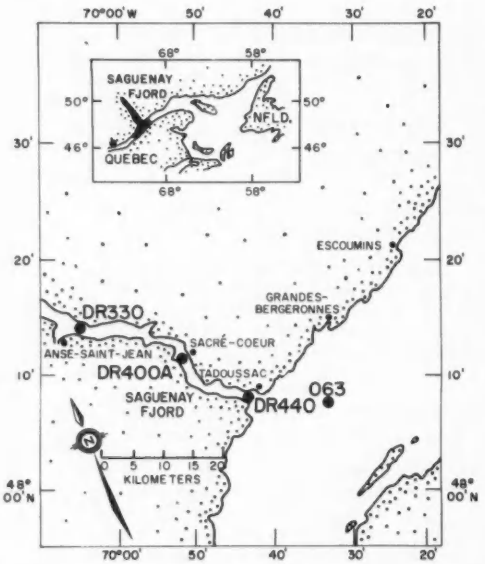

FIG. 1. Stations in the Saguenay fjord and the St. Lawrence estuary.

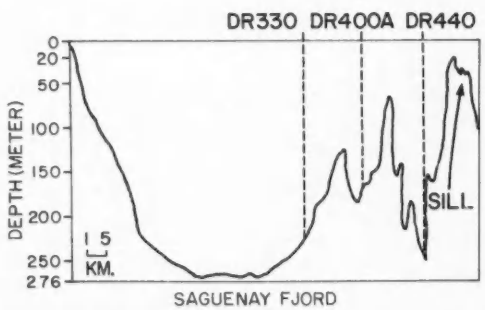

FIG. 2. Bathymetric profile of the Saguenay fjord.

mouth of the Saguenay another Station 063 (Fig. 1) was occupied.

Each of these stations was visited in June, July, August, and September 1971 at low tide or on the falling tide. In addition, in August, Station 063 was occupied for 12 h continuously.

We sampled using Niskin or Van Dorn bottles at the following depths, where the depth of the Station permitted: 0, 5, 10, 15, 25, 50, 100, and 150 m. The first subsample collected from each depth was used immediately for determination of dissolved oxygen following the method of Winkler as modified by Strickland and Parsons (1968). A second subsample was filtered immediately through a GF/C glass filter for later determination of chlorophyll *a*. The third and fourth subsamples were also filtered through GF/C paper before being frozen at −15 C for later analysis of phosphate and nitrate. A fifth subsample was drawn for salinity determination. The temperatures were obtained with bathythermographs at each Station.

Analysis for phosphate, nitrate, and chlorophyll *a* were made according to the methods described in Strickland and Parsons (1968). Salinity samples were analyzed by the inductive conductivity method using a HYTECH Model 6220 Salinometer. Both the bathythermograph and the salinity data were processed by the Canadian Oceanographic Data Centre in Ottawa.

Results and Discussion

Temperature and Salinity

As shown in Fig. 3, the summer warming of the water column at Station 063 in the St. Lawrence estuary is accompanied by progressive disappearance of the 1 and 2 C isotherms and by the deepening of the 3 and 4 C isotherms.

At Stations DR 440, DR 400A, and DR 330 (Fig. 3), the water column is divided into two distinct layers by a thermohalocline situated between 10 and 20 m. The thin, surface layer is characterized by warm, brackish water, while the deep layer consists of water of relatively low temperature and relatively high salinity. As the season advances, both temperature and salinity increase in the deep layer. The 2 C isotherm disappears progressively and the 3 and 4 C isotherms deepen, first at the mouth (Station DR 440) in June, and then further inside the fjord (Stations DR 400A and DR 330), in July and August.

From the data in Fig. 3 it can be seen that there is a fairly strong similarity between the values of temperature and salinity in the surface water at Station 063 in the estuary and those in the deep layer of the Saguenay particularly at Station DR 440. Furthermore, the temperature–salinity diagrams (Fig. 4) drawn for each month of the season using data for each Station show a strong similarity between the superficial (0–20 m) waters at Station 063 and those in the deep layer of the Saguenay. In particular, it can be seen that the density of the surface water at Station 063 is sufficient to allow it to penetrate to the deepest depth in the Saguenay fjord.

These results support the hypothesis that the warming of the deeper layer of the Saguenay results mainly from the intrusion of the relatively warmer and more saline waters of the estuary.

Dissolved Oxygen

The vertical distribution of dissolved oxygen at Station 063 is characterized by a decrease from

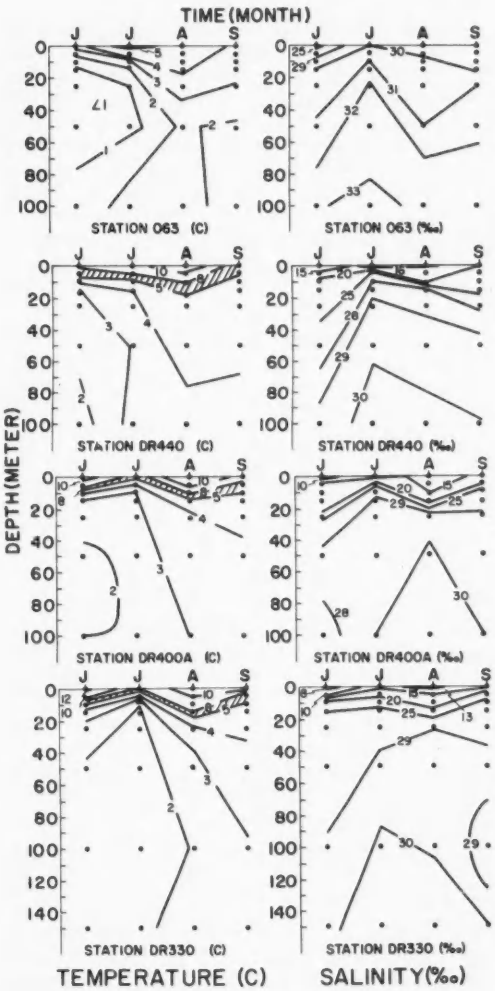

FIG. 3. Seasonal variations of isotherms and isohalines in the Saguenay and the St. Lawrence estuary. The strong thermocline in the surface waters of the Saguenay (5–8 C) is shown by the shaded area.

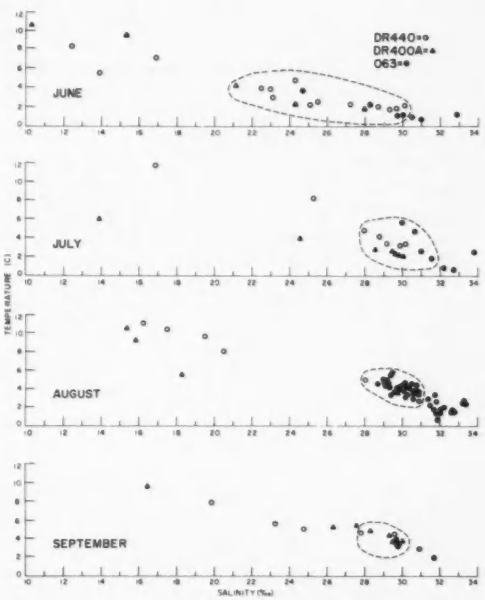

FIG. 4. Temperature–salinity diagrams for Stations DR 440, DR 400A, and DR 330 of the Saguenay, and Station 063 of the St. Lawrence estuary. The envelope includes all data from 0 to 20 m at Station 063 and from 10 m and more in the Saguenay.

the surface to the bottom. The surface waters (0–15 m) at this station are usually supersaturated; percentage O_2 saturation between 15 and 50 m is usually between 80 and 95%, i.e. the layer is relatively rich in oxygen in spite of its depth. Below 50 m percentage saturation decreases rapidly to ~50% at 100 m. A strong seasonal variation was observed in dissolved O_2 in the water column of the estuary with a maximum in July.

On the other hand, there is remarkably little variation, vertical, horizontal, or seasonal, in the O_2 content of the deep layer of the Saguenay. Indeed, taking into account all the stations sampled, the range of dissolved oxygen during the whole summer season was 0.54–0.65 mg-at/liter only, corresponding to a range of saturation from 77 to 99%. We can state then that the deep layer of the Saguenay is always well oxygenated; this is not the case in the estuary. This result confirms the observations of Drainville (1968). Finally, we note seasonal changes in dissolved O_2 in the deep layer of the Saguenay that are parallel to those in the surface layer of the estuary.

NUTRIENTS

At Station 063, two layers have been noted on the basis of nutrient concentrations. At the surface (0–50 m) concentrations of phosphate and nitrate vary between 0.5 and 1.2 μg-at PO_4-P/liter and between 5.0 and 9.5 μg-at NO_3-N/liter. In the deep layer, concentrations of phosphate and nitrate are higher (1.0–1.8 μg-at PO_4-P/liter and 7.5–13.0 μg-at NO_3-N/liter). The most interesting feature is the generally high abundance of dissolved nutrients throughout the water

column all summer long. At Station 063 an increase in nitrate and phosphate throughout the water column was observed from June to July. Concentrations remained more or less stable throughout the rest of the summer.

Vertical distribution of phosphate and nitrate in the Saguenay also showed a stratification in two layers. In the surface layer, nutrients varied from 0.2 to 0.6 μg-at PO_4-P/liter and from 1.6 to 4.9 μg-at NO_3-N/liter with an increasing gradient from the surface to the thermohalocline. Low nutrient values in this layer to be related directly to low nutrient concentrations in the surface layer further up the fjord. Concentrations of phosphate and nitrate in the deep layer are relatively high (from 0.5 to 1.0 μg-at PO_4-P/liter and from 5.0 to 9.0 μg-at NO_3-N/liter), and the vertical distribution is essentially uniform. If one compares the concentrations in the deep water of the Saguenay with those at Station 063, or indeed with any deep Station in the St. Lawrence Estuary (Therriault 1973), nutrients in the deep layer of the Saguenay are much lower; they are much closer to the surface values one finds at Station 063. Furthermore, we observed a seasonal variation in the deep layer of the Saguenay which is very similar to that in the surface layer at Station 063 in the estuary.

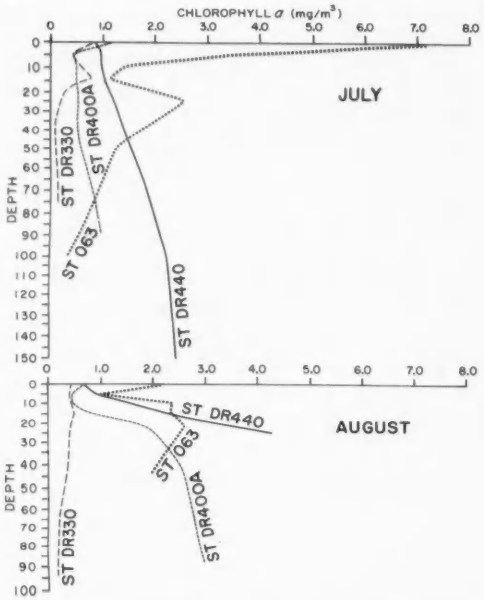

FIG. 5. Vertical distribution of chlorophyll *a* in the Saguenay and the St. Lawrence estuary, in July and August 1971.

CHLOROPHYLL

The dominant characteristic in the distribution of chlorophyll at Station 063 is its strong seasonal variability in the surface waters (0–50 m). For example, in June and September we measured values between 0.4 and 1.0 mg/m³, while in July and August we measured concentrations between 1.2 and 7.2 mg/m³ in the same layer. The maximum chlorophyll values in the surface water are found in July (Fig. 5). Seasonal variation in the deep water at Station 063 is similar to that in the surface water, but the concentrations are much lower (0.1–0.7 mg/m³).

We also observed strong seasonal variation in the surface waters of the Saguenay. This variation is different, however, from that described for Station 063 in that the maximum concentrations are found in June in the Saguenay (concentration ca. 2.5–7.6 mg/m³), compared to July and August at Station 063. In July, August, and September the concentrations in the surface waters of the Saguenay are much lower (about 0.2–0.8 mg/m³).

But the most striking characteristic of the vertical distribution of chlorophyll *a* in the Saguenay is the very high concentration in the deep layer at Stations DR 440 and DR 400A, compared to the surface layer at these same stations. Station DR 330 does not show this phenomenon (Fig. 5). It is, therefore, interesting to recall that it is also in July and August that maximum concentrations of chlorophyll in the surface waters at Station 063 are found. The strong thermohalocline that is always present in the Saguenay inhibits practically all exchange between the surface layer and the deep layer; furthermore, because the waters of this region are extremely turbid (mean extinction coefficient 0.73 [Therriault 1973]), there cannot be primary production in situ at depths greater than 10 or 15 m; advection of chlorophyll is, therefore, the only possible mechanism that would account for the observed facts.

On the strength of these observations, one could scarcely doubt that the surface waters of the estuary, because of their greater density, penetrate below the surface layer of the Saguenay during the flood tides and infiltrate the deep layer where they are essentially trapped by the shallow sill. These advected waters modify the physical, chemical, and biological characteristics of the deep layer of the Saguenay to a greater or lesser extent according to the season. Similar results have been obtained in the recent study by Bell (1973) on exchanges of the deep waters of Howe Sound, B.C., where there is also a relatively shallow sill and where strong local differences in physical–chemical properties have been found in the deep layer.

Briefly, the circulation of the Saguenay is of the estuarine type, characterized by surface transport of warm, brackish water out of the fjord during the ebb tide with entrainment of the upper part of the deep saline layer, and by the compensatory penetration during the flood tide of the surface water of the St. Lawrence estuary into the deep layer of the Saguenay.

While it is relatively simple to measure the magnitude of the surface transports from the Saguenay to the estuary by considering the mean flow, it is much more difficult to estimate quantitatively the penetration of the surface waters of the estuary into the deep layer of the Saguenay. Our data do, however, show at least an indirect indication of this deep circulation. We have already mentioned the existence of abnormally high concentrations of chlorophyll *a* in the deep layer of the Saguenay. Such values were measured at Stations DR 440 and DR 400A inside, but not at Station DR 330 where the concentrations of chlorophyll *a* are always very low in the deep layer. There is no reason to suppose the existence of a physical mechanism inhibiting exchange between DR 400A and DR 330 because we note that in the deep layer at Station DR 330, seasonal variations in physical–chemical parameters are similar to those at Stations DR 400A and DR 440. Because this chlorophyll *a* is exogenous in origin (from the surface waters of the St. Lawrence estuary), we conclude that it must travel a distance of more than 20 km from the mouth of the Saguenay before it is completely degraded. Yentsch (1965) has shown that when certain species of phytoplankton are placed in the dark for 70–100 h, their chlorophyll starts to degrade to phaeophytin, the speed of degradation is very rapid after 200 h, and the degradation is complete after 270 h. If the phytoplankton community in the deep layer of the Saguenay behaves in a similar way in the dark, we could suppose that the 20 km or so between the mouth of the Saguenay and DR 400A are traversed in less than 12 days.

If this hypothesis is sound we must attach particular importance to the mechanism of replacement of the deep waters of the Saguenay, because without doubt it occurs throughout most of the year. Evidently, because the summer warming is of only short duration in the St. Lawrence estuary (on the order of 3 mo), the summer intrusion is not extensive enough to flush the water of the previous winter out and it is probably in autumn through spring that the major influx of estuary water occurs. It is for this reason that we find at the end of September the winter waters of the previous year trapped in the deep layer at the head of the Saguenay (Drainville 1968). Pickard (1961, 1963) has already observed a similar phenomenon in certain fjords of British Columbia and he refers to it as the "memory" of the deep waters of these inlets.

The mechanism of replacement of the deep waters of the Saguenay does not exclude the second hypothesis of Drainville (1968) stating that there is simply a vertical mixing between the two layers of the Saguenay during winter. The two processes could very well go on simultaneously. Unfortunately we do not have winter (November–April) data for the Saguenay. Such measurements are essential to judge the relative importance of each of the two replacement mechanisms.

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Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature

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EMERSON, K., R. C. RUSSO, R. E. LUND, AND R. V. THURSTON. 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Res. Board Can. 32: 2379–2383.

The toxicity of ammonia to fishes has been attributed to the un-ionized ammonia chemical species present in aqueous solution. Because the percent of total ammonia present as un-ionized ammonia (NH_3) is so dependent upon pH and temperature, an exact understanding of the aqueous ammonia equilibrium is important for toxicity studies. A critical evaluation of the literature data on the ammonia–water equilibrium system has been carried out. Results of calculations of values of pK_a at different temperatures and of percent of NH_3 in aqueous ammonia solutions of zero salinity as a function of pH and temperature are presented.

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La toxicité de l'ammoniac chez les poissons a été attribuée aux type chimique d'ammoniac non ionisé présent en solution aqueuse. Comme le pourcentage d'ammoniac total présent sous forme d'ammoniac non ionisé (NH_3) dépend tellement du pH et de la température, il est important, dans des études de toxicité, de comprendre exactement l'équilibre de l'ammoniac en solution aqueuse. Nous faisons une évaluation critique des données publiées sur le système d'équilibre ammoniac-eau. Nous présentons ensuite le résultat des calculs des valeurs de pK_a à différentes température et les pourcentages de NH_3 en solutions aqueuses d'ammoniac de salinité nulle en fonction du pH et de la température.

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THE toxicity of ammonia to aquatic organisms has been attributed to the NH_3 species (Chipman 1934; Wuhrmann et al. 1947; Wuhrmann and Woker 1948); the NH_4^+ species is considered nontoxic, or significantly less toxic (Tabata 1962). It is, therefore, important to know the concentration of NH_3 in any aqueous ammonia solution in order to determine what concentrations of total ammonia are toxic to aquatic life. Current analytical methods do not permit measurement of NH_3 and NH_4^+ separately. A number of analytical methods are available for determination of total ammonia concentration in aqueous solutions, and the percent of total ammonia present as NH_3 can be determined by calculation based on the ammonia–water equilibrium.

In aqueous ammonia solutions, un-ionized ammonia exists in equilibrium with the ammonium ion and the hydroxide ion. The equation expressing this equilibrium can be written as:

$$NH_{3(g)} + nH_2O_{(l)} \rightleftarrows NH_3 \cdot nH_2O_{(aq)} \rightleftarrows NH_4^+ + OH^- + (n-1)H_2O_{(l)}$$

As indicated in this equation, the dissolved ammonia molecule exists in hydrated form; it is hydrogen bonded to at least three water molecules (Butler 1964). The dissolved un-ionized ammonia is represented for convenience simply as NH_3. The ionized form is represented as NH_4^+. The term total ammonia refers to the sum of these ($NH_3 + NM_4^+$).

The concentration of NH_3 is dependent on a number of factors in addition to total ammonia concentration. Most important among these are pH and temperature; the concentration of NH_3 increases with increasing pH and with increasing temperature. The ionic strength is the other important influence on the NH_3 concentration; there is a decrease in NH_3 concentration as the ionic strength increases in hard water or dilute saline solution. In most natural freshwater systems, even up to 200–300 mg/liter dissolved solids, the reduction of percent NH_3 attributable to dissolved solids is negligible. In more saline or very hard waters there will be small but significant decreases in the percent NH_3; these cannot be readily calculated or derived from the data presented here.

Earlier published calculations of the percent or fraction of total ammonia present as NH_3 all

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have limitations (vide infra). Some are based on inaccurate ionization constants; some have not properly considered the uncertainties in ionization constants; some have used an inappropriate interpolation process.

Methods

Four important tables giving the fraction NH_3 per total ammonia have been published previously. The first of these was Burrows (1964); his table covers a narrow range of pH and temperature. The tabulated values for the fraction of ammonia present are inaccurate. The reasons for this are unclear; no details of the calculation are given.

Trussell (1972) published a larger table covering a wide range in these two variables. Both Trussell and Burrows used the constants of Bates and Pinching (1950), with one exception; Trussell's value for the dissociation constant at 25 C differs from that given by Bates and Pinching. We do not know the source of this one constant in his data list. Trussell's values for the fraction of un-ionized ammonia are much better than Burrows'. However, Trussell's graphical method for obtaining values of K_b as a function of temperature is inherently less accurate than the digital techniques made possible by modern computers.

Two other less widely known publications in this area have appeared. Montgomery and Stiff (1971) have prepared a nomogram for determining the fraction of NH_3 at a given pH and temperature. Their nomogram is based on the most recent but not the most reliable determination of the equilibrium constant (vide infra). Finally Skarheim (1973) prepared a very extensive table giving the fraction of NH_3 as a function of pH, temperature, and total dissolved solids. However, Skarheim does not describe in any detail how his calculations were done, and statistical analysis of his reported equilibrium constants indicates that he made some questionable assumptions.

The ionization constants of the ammonium ion have been determined as a function of temperature, by Bates and Pinching (1949, 1950), using two different methods, and by Everett and Landsman (1954). An earlier determination by Everett and Wynne-Jones (1938) was later acknowledged to be in error because of a carbonate impurity in the base used in the measurements.

One can use a single one of these three sets as a data base, or combine two or three of them to make a composite set. We have chosen to use a single data set (Bates and Pinching 1949) for the following reasons. The data of Everett and Landsman seem less reliable because the method they used required liquid junctions; they were aware of this problem, but felt that their extrapolation procedure eliminated any error on this account. We are not convinced by this assertion, because all extrapolations involved data taken at single temperatures. They extrapolated their data to correct for part of this effect, but we do not think that their extrapolation would remove the effect if the liquid junction potential were temperature dependent. The fact that the results of this determination differed significantly from the other two data sets under consideration reinforces this judgment. The two determinations of Bates and Pinching (1949, 1950) are in excellent agreement, and moreover were made by two very different methods. The values of pK at a given temperature are within 1 SD at all temperatures. Both methods used by Bates and Pinching avoided the use of liquid junctions. The only significant difference in the results of their two methods is that one achieved a precision about twice that of the other; this point was discussed by Bates and Pinching (1950). The more precise of the two determinations was the earlier (1949) one, and we have accordingly used these values of pK_a in all the calculations reported below.

The three determinations discussed here represent some of the best scientific measurements of their day. Even if these measurements were repeated today, there is no reason to believe that either the accuracy or the precision of the data could be improved significantly. The question of whether the difference between the results of Bates and Pinching and the results of Everett and Landsman arises from a liquid junction potential is not experimentally resolvable. An alternate explanation of the difference is that the accuracy of the determination is inherently less than the precision obtainable, a common situation in work of this kind. The agreement obtained by Bates and Pinching using two methods of measurement is an argument against this latter suggestion, but one that should be used with great caution in defending the accuracy of their determination. We believe that the Bates and Pinching values are the best available for this constant as a function of temperature, but that the estimated uncertainties in their published values must be treated as lower limits to the true uncertainties. The results of Everett and Landsman simply underline this conclusion.

Results

Values of the constants are needed at intermediate temperatures between those for which measurements were made. Various temperature dependencies of pK_a have been discussed in the literature; we examined these in detail, but ultimately approached the problem in an empirical way. We considered the following equations as possibilities:

$$pK = A + BT \quad (1)$$
$$pK = A + B/T \quad (2)$$
$$pK = A + B/T + CT \quad (3)$$
$$pK = A + B/T + C \log T \quad (4)$$
$$pK = A + BT + CT^2 \quad (5)$$
$$pK = A + B/T + CT + DT^2 \quad (6)$$
$$pK = A + B/T + CT + D \log T \quad (7)$$

In each of these equations, $pK = -\log K_a$ (the acid dissociation constant of the NH_4^+ ion), T = temperature in degrees Kelvin, and A, B, C, and D represent empirically adjusted constants.

To decide which of these equations was most suitable to describe the data, we calculated a mean square residual (MSR) from the errors quoted by Bates and Pinching (1949) and compared it with the MSR in the fit of each of the several equations. To calculate the experimental MSR we assumed that the errors, which are indicated as "$\pm 0.000n$," represent 50% confidence intervals; this was common practice in scientific writing at that time. Hence this number is an estimate of variance rather than a MSR. The MSR's calculated for each of the equations investigated, and the experimental MSR, are as follows:

Equation no.	Mean square residual ($\times 10^6$)
1	662.0
2	1.9
3	2.0
4	2.1
5	0.48
6	0.41
7	0.82
Experimental	2.0

The most suitable equation should have a MSR comparable to that of the experimental points. If the calculated MSR is much less than the experimental, then the fit is unjustifiably good; if the former is greater than the latter, then the fit is inadequate.

This study was begun by looking at Equations (2) and (4), which represent logical starting points; the theoretical basis for both these equations has been summarized by Bates and Pinching. As can be seen from the MSR data presented above, Equation (4) actually gives a slightly worse fit than Equation (2) in spite of having more parameters in the equation. We calculated a value of 0.70 for the *t*-test (Snedecor and Cochran 1967) of the significance of this variable, indicating that the log T term contributes insignificantly to the fit.

Although the fit of Equation (2) is adequate, we were led to try Equations (3) and (5) to see if a three-parameter fit could be found that actually improved on Equation (2). Equation (5) is such an equation; the calculated MSR is about one quarter of the value for the other two three-parameter fits (Equations (3) and (4)). Equation (5) was the most difficult of the possibilities to resolve by regression analysis; the matrix of coefficients is very nearly singular, and the method of orthogonal polynomials (Snedecor and Cochran 1967) was used to obtain a solution.

Having obtained these somewhat surprising results for the three-parameter fits attempted, we decided to check some four-parameter fits to see what results these would give. Also, because our best three-parameter fit contained a linear and a quadratic term, we were interested to see whether a simple linear fit might surprise us in some way. Equation (1) represents the linear fit, and Equations (6) and (7) the four-parameter fits. Equation (1) is a very poor fit, as expected. Equation (6) is a slight improvement on Equation (5); this would be expected, because it is the same equation with a 1/T dependence added. Equation (7), which lacks the quadratic term, is worse than (5) and (6) but better than (3) and (4). However, the precise relationship among these various fits seems of little practical importance because the MSR's for Equations (5), (6), and (7) are all significantly lower than the experimentally estimated variance, and hence these equations are considered overfitted. Equation (1) is an inadequate fit and need not be considered further. Equations (2), (3), and (4) have similar MSR values, comparable to the experimental variance, but because Equation (2) is a two-parameter fit it is preferable to Equations (3) and (4) on the grounds of simplicity.

We conclude from this analysis that Equation (2) is an adequate and wholly justifiable representation of the temperature dependence of the pK for NH_3 in the interval of interest, whereas none of the other possible equations is wholly defensible. Therefore, we have used the following

TABLE 1. Calculated pK_a values for ammonia as a function of temperature (0–30 C).

Temp (C)	pK_a[a]	Temp (C)	pK_a[a]
0.0	10.0826	16.0	9.5297
1.0	10.0461	17.0	9.4972
2.0	10.0099	18.0	9.4649
3.0	9.9740	19.0	9.4328
4.0	9.9384	20.0	9.4010
5.0	9.9030	21.0	9.3693
6.0	9.8678	22.0	9.3379
7.0	9.8329	23.0	9.3067
8.0	9.7983	24.0	9.2757
9.0	9.7639	25.0	9.2448
10.0	9.7297	26.0	9.2143
11.0	9.6958	27.0	9.1839
12.0	9.6621	28.0	9.1537
13.0	9.6287	29.0	9.1237
14.0	9.5955	30.0	9.0939
15.0	9.5625		

[a]These values are significant only to three figures, but are given here to four figures which we used in subsequent calculations to minimize round-off errors.

TABLE 2. Percent NH_3 in aqueous ammonia solutions for 0–30 C and pH 6–10.

Temp (C)	pH								
	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
0	.00827	.0261	.0826	.261	.820	2.55	7.64	20.7	45.3
1	.00899	.0284	.0898	.284	.891	2.77	8.25	22.1	47.3
2	.00977	.0309	.0977	.308	.968	3.00	8.90	23.6	49.4
3	.0106	.0336	.106	.335	1.05	3.25	9.60	25.1	51.5
4	.0115	.0364	.115	.363	1.14	3.52	10.3	26.7	53.5
5	.0125	.0395	.125	.394	1.23	3.80	11.1	28.3	55.6
6	.0136	.0429	.135	.427	1.34	4.11	11.9	30.0	57.6
7	.0147	.0464	.147	.462	1.45	4.44	12.8	31.7	59.5
8	.0159	.0503	.159	.501	1.57	4.79	13.7	33.5	61.4
9	.0172	.0544	.172	.542	1.69	5.16	14.7	35.3	63.3
10	.0186	.0589	.186	.586	1.83	5.56	15.7	37.1	65.1
11	.0201	.0637	.201	.633	1.97	5.99	16.8	38.9	66.8
12	.0218	.0688	.217	.684	2.13	6.44	17.9	40.8	68.5
13	.0235	.0743	.235	.738	2.30	6.92	19.0	42.6	70.2
14	.0254	.0802	.253	.796	2.48	7.43	20.2	44.5	71.7
15	.0274	.0865	.273	.859	2.67	7.97	21.5	46.4	73.3
16	.0295	.0933	.294	.925	2.87	8.54	22.8	48.3	74.7
17	.0318	.101	.317	.996	3.08	9.14	24.1	50.2	76.1
18	.0343	.108	.342	1.07	3.31	9.78	25.5	52.0	77.4
19	.0369	.117	.368	1.15	3.56	10.5	27.0	53.9	78.7
20	.0397	.125	.396	1.24	3.82	11.2	28.4	55.7	79.9
21	.0427	.135	.425	1.33	4.10	11.9	29.9	57.5	81.0
22	.0459	.145	.457	1.43	4.39	12.7	31.5	59.2	82.1
23	.0493	.156	.491	1.54	4.70	13.5	33.0	60.9	83.2
24	.0530	.167	.527	1.65	5.03	14.4	34.6	62.6	84.1
25	.0569	.180	.566	1.77	5.38	15.3	36.3	64.3	85.1
26	.0610	.193	.607	1.89	5.75	16.2	37.9	65.9	85.9
27	.0654	.207	.651	2.03	6.15	17.2	39.6	67.4	86.8
28	.0701	.221	.697	2.17	6.56	18.2	41.2	68.9	87.5
29	.0752	.237	.747	2.32	7.00	19.2	42.9	70.4	88.3
30	.0805	.254	.799	2.48	7.46	20.3	44.6	71.8	89.0

equation to calculate pK_a at all temperatures.

$$pK_a = 0.09018 + 2729.92/T$$

It should be noted that our values for the parameters in this equation are slightly different from those reported by Bates and Pinching. We have calculated the MSR for their parameters and obtained a value of 4.1. We attribute this difference to round-off errors; Bates and Pinching carried out their calculation on an electric desk calculator, whereas we have used double precision arithmetic (approximately 15 significant fig.).

Our calculated values of pK_a are listed in Table 1.

To calculate the fraction of NH_3 using our calculated values of pK_a we have used the equation

$$f = 1/(10^{pK_a - pH} + 1)$$

Values of this fraction, expressed as percents, are listed in Table 2 at intervals of 1.0 degree from 0 to 30 C, and 0.5 pH unit from pH 6 to 10. If percent NH_3 values for smaller increments of pH and temperature are desired, these can readily be calculated using the above equations. It should be noted by future users that the equation for pK_a is invalid outside the temperature range here considered, because it was obtained empirically. Extrapolations to temperatures above 50 C or below 0 C should not be made with this equation.

All calculations were carried out using the SDS Sigma 7 computer at Montana State University. Some of these programs were written in BASIC, others in FORTRAN; these are available upon request.

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Biosynthesis of Organic Arsenic Compounds in Brown Trout (*Salmo trutta*)

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PENROSE, W. R. 1975. Biosynthesis of organic arsenic compounds in brown trout (*Salmo trutta*). J. Fish. Res. Board Can. 32: 2385–2390.

Radioactive, inorganic arsenic was administered to brown trout (*Salmo trutta*) orally and by intramuscular injection. The orally administered arsenic appeared in the tissues in an altered form that is presumed organic; this organoarsenical was also found in the gastrointestinal contents. The injected arsenic appeared in the tissues in inorganic form and was only slowly converted to organic form; meanwhile, large amounts of inorganic and organic arsenic were detected in the bile. These findings are consistent with biosynthesis of the organic arsenic compound within the gastrointestinal tract.

PENROSE, W. R. 1975. Biosynthesis of organic arsenic compounds in brown trout (*Salmo trutta*). J. Fish. Res. Board Can. 32: 2385–2390.

Nous avons administré par voie orale et par injection intramusculaire de l'arsénic inorganique radioactif dans des truites brunes (*Salmo trutta*). L'arsénic administré par voie orale apparaît dans les tissus sous une forme altérée qu'on présume organique; ce composé organo-arsénical se trouve également dans les contenus gastro-intestinaux. L'arsénic injecté apparaît dans les tissus sous une forme inorganique et ne se transforme que lentement en forme organique; entre temps, de fortes quantités d'arsénic inorganique et organique se trouvent dans la bile. Ces constatations sont en accord avec la biosynthèse du composé d'arsénic organique à l'intérieur du tractus gastro-intestinal.

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THE presence of high levels of arsenic in some marine animals has been recognized since the work of Cox in 1925. More recent investigations into the environmental biochemistries of mercury and arsenic have revealed similarities in the metabolism of the two elements (Wood 1974). Any assessment of the environmental hazard of arsenic now hinges on the characterization and toxicology of its metabolites (Penrose 1974).

As early as 1926, Chapman was able to show differences in chemical and biological properties of inorganic arsenic and the arsenic-containing extracts of marine animals. His work and that of Coulson et al. (1935) demonstrated that the large quantities of arsenic in marine crustaceans (often 100 ppm or higher) were rapidly and totally eliminated by human consumers, whereas a proportion of inorganic arsenic consumed by the same subjects was retained indefinitely. Lunde (1969) postulated the existence of organoarsenic compounds in fish based on the ion exchange behavior of extracted arsenic. It has been demonstrated in this laboratory that all of the arsenic in witch flounder (*Glyptocephalus cynoglossus*) and the shrimp *Pandalus borealis* exists as a single compound, identical in both species, that is cationic, hydrophilic, and nonvolatile (W. R. Penrose unpublished data). The structure of this compound is unknown, but based on its chemical properties it cannot be one of the known forms of environmental arsenic: methylarsines (Challenger 1945; Wood 1974), methanearsonic or dimethylarsinic acids (Braman and Foreback 1973), or inorganic arsenic.

A potential source of the arsenic found in fish is sediment particles that may be ingested with food. Sediments rarely contain less than 4 ppm of arsenic (Penrose 1974). We have demonstrated that arsenic leaches readily from sediments into sea water (Penrose et al. 1975) and ought to do so under conditions existing in the digestive tract of a fish. I therefore examined the ability of fish to convert inorganic arsenic into other forms of the element.

Materials and Methods

SPECIMENS

Brown trout (*Salmo trutta*) were trapped in Quidi Vidi Lake in St. John's, Nfld., and maintained in recirculated fresh water on a diet of beef liver.

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Animals of 200–300 g were selected for these experiments.

Chemicals

Carrier-free ^{74}As-arsenic acid was purchased from Amersham-Searle Ltd.; thin-layer chromatography of each lot revealed that 80–90% of ^{74}As was present as arsenate and the remainder as arsenite. A slight excess of solid $NaHCO_3$ was added to dilutions before administration to neutralize the HCl present. Protosol tissue solubilizer and Aquasol liquid scintillation counting fluid were purchased from New England Nuclear. Silica gel thin-layer plates (Macherey-Nagel SIL G-25) were obtained from Brinkmann Instruments Limited. A solvent system of methanol:acetic acid (9:1) adequately separated arsenate (R_f 0.8), arsenite (0–0.15), and the natural organoarsenic compound isolated from witch flounder muscle (0.35).

Biosynthesis Experiments

Fish were inspected for weight and normal appearance and removed to a separate tank. Oral administration was accomplished by injection of 1 ml of radioarsenic solution through the pharynx with a 1-ml tuberculin syringe. Intramuscular injections of 0.10 ml were made with a 22-gauge, 37-mm needle into the muscle 2 cm below the dorsal fin. Injections were always made on the left side and muscle samples removed from the right.

Fish were fed daily during experiments. At appropriate times, fish were dried with a towel and blood removed by cutting into the heart. They were then killed by decapitation. Bile was removed with a syringe and fine needle before removal of the liver. Feces and stomach contents were removed and mixed thoroughly together before sampling. Muscle samples were removed from 2 cm below the dorsal fin.

Measurement of Radioactivity

Total radioactivity in tissues was measured by immersing a weighed amount of tissue (about 0.1 g) in 2 ml Protosol in a polyethylene scintillation vial. After 24–48 h at 44 C with occasional agitation, 10 ml Aquasol and two drops acetic acid were added, and the radioactivity counted in a scintillation spectrometer.

The proportion of organic to total arsenic was estimated by a method similar to that described by Lunde (1969). A weighed amount (0.2–1.0 g) of tissue was homogenized with 4.0 ml 10% sulfosalicylic acid. After centrifugation (5 min at 7000 × *g*), 0.20 ml of the supernatant liquid was mixed with 3 ml water and 10 ml Aquasol. A second 0.20 ml of supernatant liquid was applied to a 4.5 × 50-mm column of Dowex 1-X8 or Amberlite CG-400 (200–400 mesh, hydroxide form) in a Pasteur pipette. The column was washed with 3 ml water into a scintillation vial and 10 ml Aquasol added. The ratio of the count rates in the second sample to those in the first is the proportion of nonanionic arsenic (i.e. neither arsenite nor arsenate) to total arsenic. Appropriate controls confirmed that arsenite and arsenate in the samples were retained by the anion exchanger. Total radioactivity extracted by sulfosalicylic acid was always 80–95% of that determined by Protosol solubilization. No attempt was made to wash the remaining activity from the pellet.

Where necessary, count rates were corrected for decay using a half-life of 17.7 days. The efficiency of counting was monitored by the channels ratio method; corrections were not found to be necessary at any time, however, presumably due to the high energy of the ^{74}As beta particles.

Partial Purification of Nonanionic Arsenic Compounds from Muscle

Muscle from witch flounder or trout was homogenized with an equal weight of water. To this homogenate was added an equal volume of water and 0.05 vol of 100% (wt/vol) trichloroacetic acid with stirring. After centrifugation for 10 min at 7500 × *g*, the clear supernatant was decanted. To this was added 0.025 vol of 12 N H_2SO_4, and the mixture was extracted twice with 0.2 vol of benzene. The benzene extracts were discarded. The aqueous phase was neutralized to a phenolphthalein endpoint and applied to a cation exchange column (Dowex 50-X8, 20–50 mesh, H-form, 22 × 180 mm). The column was washed with 200 ml water and eluted with 3% (vol/vol) ammonium hydroxide. Fractions containing phenolphthalein color were pooled and evaporated to dryness in vacuo at 60 C. Arsenic concentrations in various fractions were determined as described by Penrose et al. (1975).

Results

In the first experiments, small doses of ^{74}As-arsenic acid (1 μg; 20 μCi per fish) were administered intramuscularly or orally. The total tissue activities and ratios of "organic" (nonanionic) to total radioarsenic were determined (Table 1). Arsenate introduced intramuscularly appeared rapidly (4.5 h) in blood, muscle, and liver, virtually all in anionic form. Only slowly did the proportion of organic arsenic increase. When administered orally, on the other hand, the radioactivity appeared more slowly and almost entirely in organic form.

Safety considerations arising from the amount of radioarsenic that had to be administered to each fish meant that each set of data in Table 1 (one route of administration and one time point) represents only one fish. The experiment was repeated twice at appropriate intervals with qualitatively similar results. Figure 1 shows the dynamics (for liver only) of arsenic conversion in a longer term experiment.

Based on the results of this first experiment, a likely site of conversion of the arsenic to nonanionic form was the lumen of the trout gut (see

TABLE 1. Conversion of inorganic to organic arsenic by brown trout (*Salmo trutta*) after two modes of administration. Dose 1000 ng per 250 ± 50-g fish (2.25×10^7 cpm ^{74}As).

		Injected		Oral	
Time	Tissue	Concn (ng/g)[a]	Percent nonanionic	Concn (ng/g)[a]	Percent nonanionic
4.5 h	Blood	6.67	0.8	0.20	82
	Muscle	1.10	7.5	0.18	84
	Liver	2.80	5.7	1.08	93
12 h	B	2.03	6.5	0.37	96
	M	0.54	35.	0.99	97
	L	0.74	17.6	0.61	84
24 h	B	3.46	12.7	0.37	92
	M	1.05	65	2.03	97
	L	1.80	37.	1.42	105
48 h	B	0.13	26.		
	M	1.37	37.		
	L	0.76	60		

[a]Nanograms As per gram wet weight.

Discussion). Initial trials to test this hypothesis involved a) incubation of gut contents with ^{74}As-arsenate, b) incubation of isolated, ligated gastrointestinal tracts from fish with arsenate introduced into the lumen, and c) isolation of strains of aerobic bacteria from trout intestines and screening for arsenic methylation. All these approaches failed. (If methanogenic bacteria are involved, this would be expected, because these organisms require anaerobic conditions for growth. (Because these specialized techniques [Hungate 1969] were not available, the investigation was continued employing only the whole animal.)

The livers and gastrointestinal contents of trout fed tracer (carrier-free) doses of ^{74}As-arsenate were sampled after 24 and 72 h. The results of one experiment are given in Table 2. The gut contents in the four fish contained variable but significant proportions of "organic" radioarsenic.

Injected arsenic is eventually converted partly to the "organic" form. If conversion occurs solely in the gut, it is necessary that injected inorganic arsenic be excreted into the lumen. Excretion of arsenic via the bile is established in mammals (Klaassen 1974); therefore, an experiment was conducted to determine the presence of injected inorganic arsenic in the bile of trout. Six fish were administered 15 μCi each of carrier-free arsenic acid intramuscularly and killed after various intervals. The quantities of bile recoverable were in the range 0.05–0.20 ml; these were mixed with only 1.00 ml of 10% sulfosalicylic acid before determination of total and "organic" radioarsenic.

The figures in Table 3 clearly demonstrate that arsenic is rapidly excreted into the bile in both inorganic and organic forms. In the two fish sampled at 5 h most of the arsenic is inorganic and at 24 h most is organic, but in both cases the concentration in the bile is a significant proportion of that in the blood. For specimens 1, 3, 4, and 5, the calculated concentration of organic arsenic in bile is higher than in the blood, while that of inorganic arsenic is lower. (Fish 2 had too low a concentration of organic arsenic in either blood or bile to provide a meaningful datum.) Organic arsenic appears to be excreted against a concentration gradient; while inorganic arsenic is definitely excreted, the process is not necessarily an active one.

At this time, the identity of the anionic arsenic found in the blood of fishes 1 and 5 was confirmed to be inorganic. A small quantity of the acid extract was chromatographed on silica thin-layer plates with sodium arsenate and sodium arsenite. The two inorganic compounds were visualized by spraying with ammoniacal 1% silver nitrate. The plate was scraped and counted as described in the legend to Fig. 2. All of the radioactivity in fish 1 and most of that in fish 5 cochromatographed with arsenate.

CHARACTERIZATION OF NONANIONIC ARSENIC COMPOUND

Witch flounder muscle (95 g, containing 25 ppm arsenic) was processed as described in

Materials and Methods. 101% of the arsenic originally present in the muscle was recovered in the pooled fractions from the ion exchange step. Inorganic arsenic was not retained by the cation exchange resin.

From a trout that had been given an oral dose of 10 μCi ^{74}As-arsenate 24 h previously, 63 g muscle tissue was recovered. The trout muscle did not contain detectable amounts of arsenic when analyzed chemically. The yield of radioactivity eluted with ammonia from the cation exchange resin was 90%, indicating that the

TABLE 2. Organic arsenic in gastrointestinal contents and liver of trout fed ^{74}As-arsenate.

		Liver		G. I. contents	
Fish	Time	Cpm/g	Percent nonanionic	Cpm/g	Percent nonanionic
1	24 h	13800	96%	208160	72%
2	24	11520	71%	168980	52%
3	72	14880	96%	22940	16%
4	72	19940	98%	48300	75%

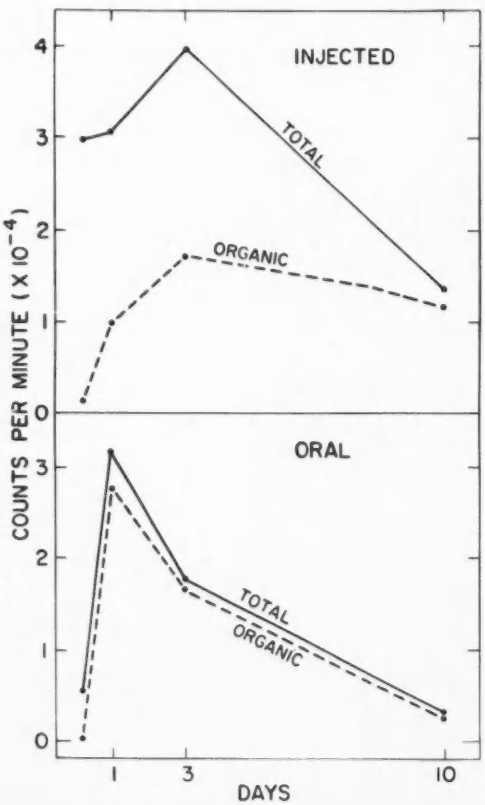

FIG. 1. Form of arsenic in the livers of brown trout (*Salmo trutta*) administered 10 μCi ^{74}As-arsenate by the intramuscular and oral routes. Liver tissue was treated as described. Counts per minute shown are for 1 g of liver tissue. Each pair of points (total and organic) represents a single fish.

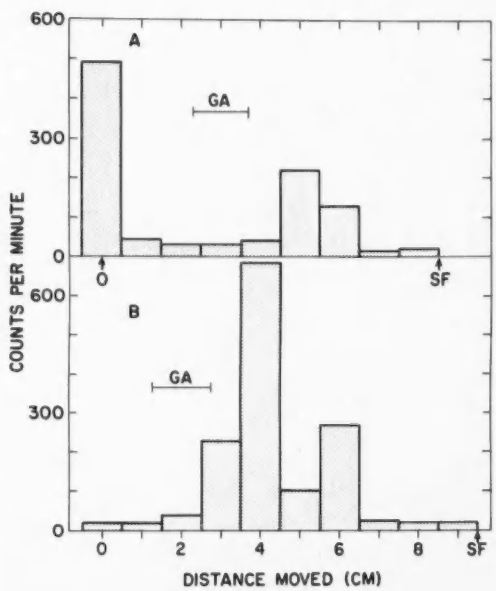

FIG. 2. Thin-layer chromatography of cationic fraction of radioarsenic extracted from trout muscle. Extracts were streaked on 20 × 20-cm Silica Gel G plates and overlaid with a sample of the witch flounder (*Glytocephalus cynoglossus*) arsenic compound. After development, 1-cm zones were scribed onto the adsorbent layer, and the layer was scribed laterally in half. One half of each 1-cm zone was scraped into a liquid scintillation counting vial; the other half was ashed for arsenic determination. Solvent systems used were (A) methanol:acetic acid (90:10) and (B) propanol:ammonia (50:1). The origin is marked as O; SF, solvent front; GA, location of witch flounder arsenical compound.

TABLE 3. Excretion of injected inorganic arsenic into the digestive tract. For clarity, proportions and concentrations are tabulated separately.

Fish no.	Time (h)	% ^{74}As in nonanionic form			
		Blood	Bile	G.I. contents	Liver
1	5	0.72	5.35	−0.47	9.74
2	5	0.44	0.23	11.0	8.30
3	24	6.30	80.3	44.9	34.7
4	24	5.36	88.0	57.9	28.9
5	72	18.3	48.3	81.7	45.7
6	72	24.1	no bile	98.5	62.1

Fish no.	Time (h)	Concn total ^{74}As (cpm/g)			
		Blood	Bile	G.I. contents	Liver
1	5	108840	22000	8640	27120
2	5	42640	14000	1200	10840
3	24	44000	17000	35200	18760
4	24	23500	3250	1420	8360
5	72	24000	9900	6460	9600
6	72	24400	no bile	5080	10500

nonanionic arsenic was similar to that in witch flounder at least in its hydrophilic and cationic properties. Thin-layer chromatography revealed that two compounds were present in the eluate, neither of which was identical to that isolated from witch flounder (Fig. 2).

Discussion

Very little is now known of the metabolism of arsenic outside of the simple alkylations performed by certain bacteria, fungi, and yeasts (Challenger 1945; McBride and Wolfe 1971; Cox and Alexander 1973). Lunde has observed transformations of arsenic in unicellular algae (1973) and rainbow trout (1972). The products of these transformations he described as "organoarsenic" compounds, but there is as yet no evidence that the compounds actually contain carbon–arsenic bonds. The term "organic" must therefore be applied cautiously when describing these compounds.

Lunde (1972) administered radioactive inorganic arsenic to trout by addition to the water and by feeding. Arsenic was only weakly absorbed from water and rapidly lost on return to clean water. He observed, as I have, that arsenic fed to the fish is converted to "organic" form. The present work has demonstrated the following: a) arsenic introduced via the gastrointestinal tract was much more efficiently converted to the organic form than arsenic introduced systemically; b) nonanionic arsenic appeared within 1 day in the gastrointestinal tract of fish fed inorganic arsenic; the nonanionic arsenic seemed to be selectively absorbed; c) both inorganic and nonanionic arsenic were excreted in the bile. The potential therefore exists for the cycling of arsenic between the body and the lumen of the intestine. The apparently selective absorption of nonanionic arsenic insures that the proportion of arsenic in the tissues will shift towards the nonanionic form, regardless of the route of administration. These findings are consistent with the hypothesis that the conversion to "organic" form is mediated by the intestinal flora.

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Heavy Metal and Chlorinated Hydrocarbon Residues in California Sea Lions (*Zalophus californianus californianus*)[1]

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BUHLER, D. R., R. R. CLAEYS, AND B. R. MATE. 1975. Heavy metal and chlorinated hydrocarbon residues in California sea lions (*Zalophus californianus californianus*). J. Fish. Res. Board Can. 32: 2391–2397.

Samples of various tissues and organs from healthy California sea lions (*Zalophus californianus californianus*) and sick animals (apparently with leptospirosis) collected along the central Oregon coast in 1970, 1971, and 1973 were analyzed for total mercury, methylmercury, cadmium, and chlorinated hydrocarbons. Maximum mercury concentrations of 74–170 ppm occurred in sea lion liver, but only 1.6–3.7% of this was present as methylmercury. Cadmium was concentrated primarily in the kidney which contained 7.2–12.0 ppm of the metal. Chlorinated hydrocarbon residues in sea lion fat ranged between 253–475 ppm DDE, and 21.2 and 34.1 ppm PCB. Although mercury, cadmium, and chlorinated hydrocarbon residues in some of the sick sea lions were significantly higher than those present in healthy animals, it is not possible to relate these differences to the onset of leptospirosis.

BUHLER, D. R., R. R. CLAEYS, AND B. R. MATE. 1975. Heavy metal and chlorinated hydrocarbon residues in California sea lions (*Zalophus californianus californianus*). J. Fish. Res. Board Can. 32: 2391–2397.

Nous avons dosé le mercure total, le mercure-méthyl, le cadmium et les hydrocarbures chlorés dans des échantillons de divers tissus et organes d'otaries de Californie (*Zalophus californianus californianus*) en santé et d'otaries malades (apparamment atteintes de leptospirose) capturées le long de la côte de l'Orégon central en 1970, 1971 et 1973. Les concentrations maximales de mercure de 74–170 ppm se rencontrent dans le foie, mais seulement 1.6–3.7% sous forme de mercure-méthyl. Le cadmium est concentré surtout dans le rein qui contient 7.2–12.0 ppm de ce métal. Les résidus d'hydrocarbures chlorés dans des otaries grasses sont de 253–475 ppm de DDE et de 21.2–34.1 ppm de BPC. Quoique les niveaux de mercure, de cadmium et de résidus d'hydrocarbures chlorés chez quelques-unes des otaries malades soient plus élevés que ceux des animaux sains, il nous a été impossible d'établir une relation entre ces différences et le début de la leptospirose.

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IN the fall and winter of 1970, male California sea lions, *Zalophus californianus californianus*, were found in large numbers from southern California to the Columbia River in atypical locations, particularly in or near freshwater. Many of these animals were sick or had died. Vedros et al. (1971) obtained convincing microscopic and serologic evidence suggesting that the etiologic agent for this epizootic among California sea lions was a species of *Leptospira*. Detailed examination of the tissues on necropsy showed that all the animals suffered from severe nephritis and that the kidney was the only organ with significant histopathologic lesions.

To determine whether tissue concentrations of various environmental contaminants were significantly altered during this serious die-off, we examined the total mercury, methylmercury, cadmium, chlorinated hydrocarbon, and polychlorinated biphenyl (PCB) content of various tissues and organs from sick and healthy California sea lions.

Materials and Methods

During 1970, recently dead California sea lions were found along the Oregon coast. In addition, in

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1970, 1971, and 1973 under permits issued by the Oregon Game Commission or the National Marine Fisheries Service, sick or healthy sea lions from the same areas were humanely killed by shooting. Selected tissues were then removed from these animals, packaged in individual plastic bags, and frozen for subsequent analysis. The ages of the animals were determined by counting dental annuli in sectioned and polished canine teeth using the replica method of B. R. Mate and W. Orr (unpublished data).

For cadmium and total mercury analyses, tissues were digested with a 1:1 H_2SO_4–HNO_3 mixture as described by Buhler et al. (1973). Mercury was determined by flameless atomic absorption using a Coleman Model 50 mercury analyzer. After ammonium pyrrolidine dithiocarbamate chelation and extraction from the digestion mixture with methyl isobutylketone, cadmium was measured in a Perkin-Elmer Model 403 atomic absorption spectrometer equipped with a Model HGA-2000 graphite furnace. Methylmercury was extracted from tissues essentially by the method of Thom and LeFavoure (1971) and analyzed by electron capture gas chromatography on a 5% Hi-Eff. 10B (Applied Science Laboratories, State College, Pa.) column at 170 C in a Varian Aerograph Model 2100 or Hi-Fi gas chromatograph (Kamps and McMahon 1972).

Chlorinated hydrocarbons were separated following the procedure reported by Claeys et al. (1975). Tissues were extracted with 1:1 hexaneacetone and the lipid removed by acetonitrile–water elution from an adsorption column (Giuffrida et al. 1966). Additional cleanup was achieved by Florisil chromatography. Separation of the PCBs from the other chlorinated hydrocarbons was obtained by hexane elution from a 1% water deactivated silicic acid column similar to that employed by Armour and Burke (1970). Electron capture gas chromatography was carried out on QF-1, methyl silicone, and DEGS columns using a Varian Aerograph Model 1440 or Hi-Fi gas chromatograph. For quantification of the PCBs, commercial mixtures (Aroclor 1254 and 1260) were used as references and the heights of the four largest peaks in the sea lion extract were used for calculation.

Data were analyzed for statistical significance by a computer adaptation (Guthrie et al. 1973) of the standard *t*-test.

Results and Discussion

During the fall of 1970, we made a collection of 10 male[4] sea lions, ages ranging from 5 to 9 yr, from along the central Oregon coast. The seven animals still alive exhibited extreme lethargy and a reluctance to use the hind flippers. These animals also possessed abnormally high white blood counts and exceptionally high blood urea nitrogen (BUN) values (B. R. Mate unpublished data). The only obvious lesion was the pale white appearance of the kidneys in contrast to their normal mottled purple and red color. The reluctance to use the hind limbs, the unusual consumption of fresh water, the high BUN values, and the gross appearance of the kidneys suggested kidney insufficiency which is consistent with the diagnosis of leptospirosis (Vedros et al. 1971). For comparative purposes a collection was made in the fall and winter 1971 from the same areas, consisting of two sea lions (ages 5 and 6) that exhibited similar behavioral, clinical, and postmortem symptoms to the sick animals collected during 1970, and six active sea lions (ages 4–8) that appeared healthy and possessed normal blood profiles. One of the seemingly healthy animals (specimen No. 18), however, appeared to have a liver disease, since many of the tissues were colored green. A second collection of three healthy males (ages 4–6) from the same area was also made in October 1973.

Mercury

The distribution of mercury in the two groups of healthy sea lions was quite similar (Table 1). Highest mercury concentrations occurred in the liver and ranged from 49 to 120 ppm, with a mean of 74.1 ppm for animals collected in 1971 and 95.7 for animals taken in 1973. Mercury levels in the other tissues examined were substantially lower, falling between 0.11 ppm for fat and 6.96 ppm for kidney. In the limited age range examined, there did not appear to be any age-related increase in tissue mercury levels as observed by Anas (1974) for northern fur seals.

Mercury concentrations in the liver and muscle of sick sea lions collected in 1970 were significantly higher than those of healthy animals (Table 1). Liver concentrations in the sick animals ranged from 3.18 to 240 ppm (mean 170 ppm). Sick animals taken in 1971 also contained significantly higher concentrations of hepatic mercury compared to the healthy sea lions. Although the differences were significant only at the 10% level, mercury concentrations in the kidneys of the sick 1970 sea lions were appreciably lower than those observed in the healthy animals.

The methylmercury content of different tissues from healthy and sick sea lions varied between 1.57 and 102.6% and 2.0 and 98.5% of the total mercury, respectively (Table 1). The smallest percentages of methylmercury were found in the liver (1.57–3.66%) and the kidney which contained somewhat higher concentrations (17.2–26.2%). The mercury present in the remaining

[4]Only male *Zalophus* migrate northward after the breeding season from California along the coast of Oregon, Washington, and British Columbia.

TABLE 1. Distribution and form of mercury in healthy and sick California sea lions (*Zalophus californianus californianus*).[a]

Tissue	Year	Total mercury (*ppm*)	Methylmercury[b] (*ppm*)	Methylmercury (%)
Healthy animals				
Liver	1971	74.1 ±17.2 (5)	2.59±0.83 (5)	3.66± 1.48
	1973	95.7 ±24.5 (3)	1.50±0.66 (3)	1.59± 0.64
Kidney	1971	6.96± 4.84 (5)	0.73±0.28 (5)	17.2 ±15.9
	1973	5.43± 4.74 (3)	0.66±0.43 (3)	17.5 ± 9.0
Muscle	1971	1.23± 0.53 (6)	1.17±0.40 (5)	88.6 ±12.7
	1973	0.84± 0.50 (3)	0.84±0.44 (3)	102.6 ±10.7
Heart	1971	0.59± 0.23 (5)	0.51±0.16 (5)	88.1 ±13.0
	1973	0.51± 0.34 (3)	0.32±0.22 (3)	61.9 ± 3.6
Cerebellum	1971	0.53± 0.22 (3)	0.35±0.16 (2)	59.4 ± 7.1
	1973	0.36± 0.15 (3)	0.15±0.07 (3)	40.5 ± 6.3
Cerebrum	1971	0.48 (1)	—	—
	1973	0.66± 0.48 (3)	0.14±0.06 (3)	28.5 ±18.9
Fat	1971	0.19± 0.09 (6)	—	—
Sick animals[c]				
Liver	1970[d]	170.1 ±67.2 (8)[e]	3.04±1.44 (8)	2.00± 1.32
	1971	127.5 ± 4.95 (2)[e]	2.92±1.77 (2)	2.30± 0.99
Kidney	1970	3.76± 1.35 (8)	0.90±0.35 (8)	26.2 ±11.9
	1971	4.57± 1.82 (2)	0.79±0.01 (2)	18.7 ± 7.6
Muscle	1970	1.64± 0.49 (9)[f]	1.39±0.24 (8)	92.8 ± 7.4
	1971	1.38± 0.13 (2)	1.36±0.15 (2)	98.5 ± 1.13
Heart	1970	0.80± 0.20 (5)	0.70±0.16 (5)[f]	88.2 ±12.1
	1971	0.95± 0.18 (2)	0.72±0.16 (2)	75.3 ± 2.3
Cerebellum	1970	0.90± 0.36 (3)	0.68±0.25 (3)	74.9 ± 6.9
Cerebrum	1970	0.87± 0.32 (8)	0.49±0.16 (7)	56.2 ±10.5
Fat	1970	0.17± 0.10 (9)	—	—
	1971	0.16± 0.05 (2)	—	—

[a]Mean ppm (wet weight) ±SD. Number of animals examined in parentheses.
[b]Corrected for 95% recovery.
[c]Data from animal no. 4 (1970) was omitted because of an extremely low concentration of mercury in the liver (3.18 ppm, 43.7% methylmercury), although other tissues were within the range of the other sick animals.
[d]A portion of the total mercury data for 1970 sick animals was previously reported (Buhler and Mate 1973).
[e]Significantly different from 1971 healthy animals at $P < 0.01$.
[f]Significantly different from 1971 healthy animals at $P < 0.05$.

tissues was primarily in the methylmercury form. Significantly greater methylmercury concentrations were found only in the heart of the 1970 sick sea lions as compared to the 1971 healthy animals.

High levels of mercury have also been found in harbor porpoises (Gaskin et al. 1972; Heppleston and French 1973; Freeman and Horne 1973), fur seals (Anas 1974a), gray seals (Heppleston and French 1973; Freeman and Horne 1973), harbor seals (Gaskin et al. 1973; Anas 1974a), pilot whales (Gaskin et al. 1974), and harp seals (Freeman and Horne 1973). As with other vertebrates exposed to methylmercury (Aberg et al. 1969), the highest mercury residues occur in the livers of marine mammals. Maximum hepatic mercury levels found were 182 ppm in gray seals (Heppleston and French 1973) and 249 ppm in the 1971 sick California sea lions from the present study. By contrast, mercury concentrations in the liver of female *Zalophus* collected in southern California in the winter of

TABLE 2. Distribution of cadmium in healthy and sick California sea lions.[a]

Tissue	Healthy animals		Sick animals	
	1971	1973	1970	1971
Liver	2.29 ±2.23 (4)	1.61 ±0.16 (3)	2.35 ±1.17 (7)	3.46 ±0.28 (2)
Kidney	12.0 ±4.9 (5)	7.22 ±2.71 (3)	9.70 ±3.98 (7)	11.5 ±7.3 (2)
Muscle	0.16 ±0.10 (5)	0.085±0.013 (3)	0.076±0.033 (7)	0.069±0.03 (2)
Heart	—	0.14 ±0.05 (3)	—	—
Cerebellum	0.079±0.054 (2)	0.032±0.002 (3)	0.061 (1)	—
Cerebrum	0.042±0.024 (2)	0.032±0.006 (3)	0.17 ±0.13 (4)[b]	—
Fat	—	0.043±0.015 (3)	—	—

[a]Mean ppm (wet weight) ±SD. Number of animals examined in parentheses.
[b]Significantly different from 1971 healthy animals at $P < 0.05$.

1970 were only 36–64 ppm (DeLong et al. 1973).

Although mercury levels in the liver of the sick sea lions were significantly higher than those present in normal animals on the basis of the present studies (Table 1), it is not possible to establish whether such residues contributed to the onset of leptospirosis. The discovery of mercury concentrations of 700 ppm (Anas 1974a) and 765 ppm (Koeman et al. 1973) in the livers of apparently normal harbor seals suggests that marine mammals can safely tolerate even higher concentrations of the metal. The significantly higher mercury residues in the livers of sick animals could result from the nephritis and apparent reduction in bile flow in animals afflicted with leptospirosis (Vedros et al. 1971).

One of the most interesting aspects of the present study is that 97–98% of the mercury in sea lion liver was in the inorganic form. Northern fur seals (D. R. Buhler and R. E. Anas unpublished data), gray and harp seals (Freeman and Horn 1973), and harbor seals (Gaskin et al. 1973) show similar high concentrations of inorganic mercury in the liver. Analysis of the stomach contents of two California sea lions from the present study, however, showed that about 85% of the 0.50 ppm mercury present was in the methylmercury form. In addition, marine fishes similar to those that serve as the principal diet of carnivorous marine mammals also contain primarily methylmercury (Norén and Westöö 1967; Buhler et al. 1973). Therefore, it would appear that some marine mammals, including the California sea lion, have the capacity to convert dietary methylmercury to inorganic mercury. The fact that the liver contains the highest percentages of inorganic mercury suggests that the demethylation of methylmercury may occur in that tissue. Although total mercury levels in the liver of healthy California sea lions are considerably higher than those found in the livers of human victims of the Minamata Bay poisoning (Takeuchi 1972), the apparent insensitivity of marine mammals to high mercury concentrations may result from the relatively low percentage of methylmercury present in the liver of the latter group.

CADMIUM

The concentrations of cadmium in tissues from the four groups of sea lions were similar (Table 2). Tissue cadmium levels ranged from 0.032 to 12.0 ppm with the highest concentrations occurring in the kidney. Only in the cerebrum of sick sea lions were cadmium levels significantly higher than in healthy animals.

Cadmium also accumulates in the tissues of other species of carnivorous marine mammals. Unlike mercury, however, cadmium is concentrated mainly in the kidneys. Kidney concentrations of 22 ppm for gray seals (Heppleston and French 1973) and 15.6 ppm for fur seals (Anas 1974a) have been reported. Since cadmium concentrations in the kidney of California sea lions were somewhat lower than in other marine mammals, and since there did not appear to be any great differences between concentrations present in healthy and sick animals, it appears unlikely that this metal was a contributing factor in the leptospirosis epizootic among *Zalophus*.

CHLORINATED HYDROCARBONS

Appreciable concentrations of DDT, its breakdown products, and PCBs were also present in sea lion tissue, especially in the liver and fat (Table 3). Except in the liver, DDE and PCB concentrations were of similar magnitude in the tissues of normal and sick sea lions. DDE concentrations in the liver of the 1970 sick sea lions, however, were significantly higher than those of the 1971 healthy animals, while PCB levels were significantly lower in the sick animals.

TABLE 3. Distribution of DDE and PCBs in healthy and sick California sea lions[a]

Tissue	Year	Lipid (%)	DDE (*ppm*)[b]	PCB (*ppm*)[b]
Healthy animals				
Liver	1971	2.42± 0.94 (6)	11.1 ± 3.6	4.87± 4.51
	1973	8.0 ± 0.4 (3)	12.9 ± 4.1	2.39± 1.06
Muscle	1971	0.54± 0.38 (6)	3.12± 1.43	0.70± 0.79
	1973	4.6 ± 0.4 (3)	2.65± 0.72	0.36± 0.21
Cerebrum	1971	11.8 ± 2.5 (5)	9.16± 3.28	2.82± 1.90
	1973	17.2 ± 3.8 (3)	3.02± 1.09	0.49± 0.20
Fat	1971	41.6 ±25.3 (6)	253 ±116	34.1 ±20.8
	1973	64.7 ± 6.8 (3)	342 ±234	21.2 ± 2.1
Sick animals				
Liver	1970	4.23± 1.36 (8)	23.9 ±18.9[c]	2.79± 1.84[c]
	1971	2.85± 0.81 (2)	11.2 ± 0.3	1.95± 0.64
Muscle	1970	0.58± 0.32 (8)	3.20± 1.42	0.32± 0.21
	1971	1.16± 0.27 (2)	5.70± 1.44	0.86± 0.23
Cerebrum	1970	13.5 ± 3.14 (5)	8.27± 2.86	1.60± 0.73
Fat	1970	56.4 ±10.6 (7)	358 ±213	24.2 ±11.5
	1971	35.3 ±27.4 (2)	475 ±311	28.5 ± 2.1

[a]Mean value ±SD. Number of animals examined in parentheses.
[b]On a wet weight basis.
[c]Significantly different from 1971 healthy animals at $P < 0.05$.

TABLE 4. Distribution of DDT and its metabolites in tissues from healthy and sick California sea lions.[a,b]

Tissue	Lipid (%)	*p,p'*-DDE (%)	*p,p'*-DDD (%)	*p,p'*-DDT (%)	Total DDT residue[c] (*ppm*)
Healthy animals (1971)					
Liver	2.19± 0.86 (5)	95.7±4.2	3.28±2.98	1.00±1.31	11.6 ± 4.4
Muscle	0.54± 0.38 (2)	94.3±0.1	4.45±0.49	1.25±0.35	3.24± 1.49
Cerebrum	12.5 ± 0.6 (2)	93.3±0.7	4.10±0.42	2.55±0.35	7.54± 3.35
Fat	23.0 ±14.5 (2)	92.7±3.0	4.10±1.13	3.25±1.77	199 ±128
Sick animals (1971)					
Liver	2.85± 0.81 (2)	97.6±1.6	1.65±1.34[d]	0.40±0.14	11.4 ± 0.1
Muscle	1.15± 0.25 (2)	96.9±1.3	2.56±0.52	0.60±0.85	5.89± 1.56

[a]Mean value ±SD. Number of animals examined in parentheses.
[b]The 1973 healthy animals contained 96.9 ± 0.7% *p,p'*-DDE and 3.15 ± 0.73% *p,p'*-DDD in cerebellum and 97.7 ± 0.3% *p,p'*-DDE and 2.27 ± 0.34% *p,p'*-DDD in the cerebrum. No other chlorinated hydrocarbons were detected in these tissues.
[c]On a wet weight basis.
[d]Significantly different from 1971 healthy animals at $P < 0.05$.

Other than *p,p'*-DDT, its metabolites and PCBs, no other chlorinated hydrocarbon pesticides were detected in this study. *p,p'*-DDE was the principal DDT metabolite present in sea lion tissues, accounting for 92.7–97.9% of the total DDT residue present in the 1971 collection (Table 4). The remaining DDT residue consisted of 1.65–4.45% *p,p'*-DDD and 0.40–3.25% *p,p'*-DDT. Only in the liver of sick animals was the concentration of *p,p'*-DDD significantly lower than that found in the healthy animals from 1971. The percentage distribution of DDT and its metabolites in the Oregon animals was similar to that observed by Le Boeuf and Bonnell (1971) for *Zalophus* collected from southern California. Only small amounts of *p,p'*-DDD were detected in addition to *p,p'*-DDE and the PCBs in the healthy animals from 1973.

Organochlorine pesticides and PCB levels are also known to be high in carnivorous marine

mammals. Because of their lipophylic nature, these persistent environmental contaminants tend to accumulate in tissues with high lipid contents so that the highest tissue residues are found in body fat. For example, high concentrations of DDE, and in some cases, the PCBs have been detected in the fat of harbor porpoises (Holden and Marsden 1967; Gaskin et al. 1973), fur seals (Anas and Wilson 1970), harbor seals (Gaskin et al. 1971; Shaw 1971; Anas 1974b), gray seals (Holden and Marsden 1967), and whales (Wolman and Wilson 1970; Gaskin et al. 1974). Maximum concentrations of DDE and PCBs found in fat of the above species were 181 and 240 ppm, respectively.

Concentrations of chlorinated hydrocarbons are generally much higher in healthy California sea lions (Le Boeuf and Bonnell 1971; DeLong et al. 1973; Table 3) than those reported for other species of marine mammals. Although male California sea lions move northward along the Pacific coast after the breeding season, they spend much of the year (as do females), in the waters off southern California. Since marine organisms in that area are highly contaminated with the PCBs and DDT and its metabolites from the Los Angeles sewage discharge (MacGregor 1974), it seems likely that the high chlorinated hydrocarbon residues in California sea lions are derived from that source. As a result of controls on industrial discharge, DDE residues in the marine biota in the vicinity of Los Angeles have started to decline in the past few years (MacGregor 1974). Nevertheless, DDE levels in healthy sea lions were higher in 1973 than in 1971 (Table 3). No DDT was detected, however, in the 1973 samples (Table 4), suggesting that the pesticide residues were not newly introduced into the environment.

The fat of healthy male and female California sea lions collected along the southern California coast between September and December 1970 contained total DDT residues from 41 to 1929 ppm while comparable values in male animals apparently sick with leptospirosis (Vedros et al. 1971) were 689–1006 ppm (Le Boeuf and Bonnell 1971). Le Boeuf and Bonnell (1971) concluded that the high DDT residues did not directly cause the increased mortalities then being observed among California sea lions.

Similar conclusions were reached in the present studies. Although there were some differences in the livers of the 1970 animals, the DDE and PCB concentrations in the tissues of sick or dead California sea lions from the Oregon coast were comparable to those found in healthy animals (Table 3). There is, therefore, no direct evidence that the accumulation of high chlorinated hydrocarbon residues played any role in the 1970–71 leptospirosis epizootic. De Long et al. (1973), however, suggested that high DDT residues in female California sea lions affected the survival of newborn pups.

The DDE and PCB concentrations observed in California sea lions collected from the Oregon coast during October–December 1970 (Table 3) were one half to two thirds lower than those found in sea lions taken along the southern California coast at about the same time (Le Boeuf and Bonnell 1971). Since DDE and PCB contamination of marine fishes is known to be very high in southern California water (MacGregor 1974), these differences may have reflected the higher dietary intake of chlorinated hydrocarbons for sea lions in southern California waters as opposed to those that seasonally migrate to the Oregon coast. It is also possible that fat stores could have become somewhat depleted during the northward migration of sea lions, perhaps causing a loss of stored chlorinated hydrocarbons. This conclusion is supported by the somewhat reduced lipid content of body fat found in the sick 1970 animals (41.6%), and healthy 1971 (56.4%) and 1973 (64.7%) animals, as compared to lipid contents of 67.4% for sick animals and 74.3% for healthy animals found in the sea lions collected off southern California by Le Boeuf and Bonnell (1971).

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Reproduction, Maturation, and Identification of Larvae of some *Sebastes* (Scorpaenidae) Species in the Northeast Pacific Ocean

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WESTRHEIM, S. J. 1975. Reproduction, maturation, and identification of larvae of some *Sebastes* (Scorpaenidae) species in the northeast Pacific Ocean. J. Fish. Res. Board Can. 32: 2399–2411.

Insemination and parturition seasons, maturation, and descriptions of preextrusion larvae were reported for several *Sebastes* (Scorpaenidae) species (66,760 specimens; 31 species) studied during 1963–73 in the northeast Pacific Ocean, from Oregon (43°N) to the western Gulf of Alaska (170°W). Principal month of insemination was October. Parturition season varied considerably among species — earlier than February for two species, and later than June for five. Within species, parturition season was generally later in the higher latitudes. Maturation of both sexes included an intermediate stage whose minimum duration was 1 yr. Length–maturity relationships were determined for 18 *Sebastes* species. The size interval in which 50% of specimens were mature ranged from <17 cm for male *S. saxicola* to 48 cm for female *S. pinniger*. Identification of preextrusion larvae by conventional morphological criteria was precluded by interspecific similarities and intraspecific differences. Within-area bathymetric variation in gonad development of *S. alutus* provided additional evidence of subpopulations.

WESTRHEIM, S. J. 1975. Reproduction, maturation, and identification of larvae of some *Sebastes* (Scorpaenidae) species in the northeast Pacific Ocean. J. Fish. Res. Board Can. 32: 2399–2411.

Les saisons d'insémination et de parturition, la maturation et les larves avant expulsion sont décrites pour plusieurs espèces de *Sebastes* (Scorpaenidae) (66,760 spécimens; 31 espèces) étudiées entre 1963 et 1973 dans le nord-est du Pacifique, de l'Orégon (43°N) à la partie ouest du golfe d'Alaska (170°W). Octobre est le principal mois d'insémination. La saison de parturition varie considérablement d'une espèce à l'autre — plus tôt que février pour deux espèces et plus tard que juin pour cinq. Dans une même espèce, la saison de parturition est généralement plus tardive dans les hautes latitudes. La maturation chez les deux sexes comporte un stade intermédiaire d'une durée minimale de 1 an. Nous avons déterminé les relations longueur–maturité chez 18 espèces de *Sebastes*. L'intervalle de longueur à laquelle 50% des spécimens sont matures varie de 17 cm pour *S. saxicola* mâle à 48 cm pour *S. pinnifer* femelle. Des similarités interspécifiques et des différences intraspécifiques nous ont empêché d'identifier, par des critères morphologiques conventionnels, les larves avant expulsion. La variation bathymétrique, dans une même région, du dévelopement des gonades de *S. alutus* fournit une preuve additionnelle de l'existence de sous-populations.

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THE ovoviviparous reproductive cycle of *Sebastes* is well established for Atlantic (Schmit 1944; Magnusson 1955; Sorokin 1958, 1961) and Pacific (Eigenmann 1893; Harada 1962; Moser 1967a, b; Sorokin 1967) forms, and consists of three phases — insemination, fertilization, and parturition. Insemination usually occurs in autumn, fertilization in winter or spring, and parturition about 1–2 mo after fertilization. Considerable variation in the timing of this cycle has been noted among the few species for which information is available (Hitz 1962; DeLacy et al. 1964). Little is known about the identification of pre- and postextrusion larvae.

During 1967–73, I undertook a detailed investigation into the reproductive cycle and identification of *Sebastes* larvae off British Columbia and southeastern Alaska (47°20′ to 58°N). Primary emphasis was placed on those species inhabiting the outer continental shelf and upper continental slope. Preliminary results of the 1967–70 collections have been published (Westrheim

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TABLE 1. Gonad-condition criteria applied to *Sebastes* specimens collected during 1967–1973. (Corresponding USSR criteria in parentheses.)

Code		Condition	Description
			Males
1	(I)	Immature	Stringlike, translucent
9	(–)	Maturing	Stringlike, slight swelling, translucent white
8	(II–VI)	Mature	
A	(III)	Resting	Ribbonlike, small, brown
B	(IV)	Developing	Ribbonlike, swelling, brown–white
C	(V)	Developed	Large, white, easily broken
			Females
1	(I)	Immature	Small, translucent
2	(II)	Maturing	Small, yellow,[a] translucent or opaque
3	(III–IV)	Mature	Large, yellow,[a] opaque
4	(V)	Fertilized	Large, orange–yellow,[a] translucent
5	(VI–VII)	Ripe	Large, translucent yellow or gray, with black dots (contain embryos or larvae)
6	(VIII)	Spent	Large, flaccid, red. A few larvae may be present
7	(–)	Resting	Moderate size, firm, red–gray, some with black blotches

[a]During this study, the following species were found to possess cream-colored ovaries: *aleutianus*, *borealis*, *brevispinis*, *crameri*, *entomelas*, and *paucispinis*.

et al. 1968; Westrheim et al. 1968; Harling et al. 1969; Harling et al. 1971). This report summarizes the results of all such studies conducted at the Pacific Biological Station during 1963–73, and deals with time of insemination and parturition, size (age) at maturity, and identification of pre- and postextrusion larvae.

Materials and Methods

Collection of Samples

Samples were collected throughout the northeast Pacific Ocean, from Oregon (43°N) to the western Gulf of Alaska (170°W). During 1963–66 limited sampling, primarily of *S. alutus*, took place throughout the study region, while during 1967–73, relatively intensive sampling, involving numerous species, took place off British Columbia and in the Southeast Gulf.

Most of the specimens were caught by on-bottom trawl and processed aboard the R/V *G.B. Reed*. The remainder were collected from off-bottom trawl catches (*G.B. Reed* and others), commercial trawl landings in British Columbia, and angling catches.

Processing

All specimens were measured to the nearest lower centimeter, fork length. Gonad-condition code used during 1963–66 was that of Westrheim (1958), and contained three categories for testes (immature, maturing, and mature) and six categories for ovaries (immature, maturing, mature, ripe, spent, and resting). For the 1967–73 collections, five categories were created for testes, and seven categories for ovaries (Table 1). Corresponding condition-code numbers used by USSR scientists (based on the work of Sorokin) are included in Table 1, and were kindly provided by Dr Yu. K. Yermakov (Pacific Ocean Scientific Research Institute of Fisheries and Oceanography, Vladivostok).

Most larvae collected from ripe females were fixed in 4% formalin for description ashore. Some were placed in shipboard aquaria (Westrheim et al. 1968). Each lot occupied a separate aquarium. Samples were collected, and fixed, from each aquarium at daily intervals until all larvae had perished. Larvae were not fed.

Descriptions of larvae were prepared using the morphological criteria of DeLacy et al. (1964). Specimens were usually measured total length, to the nearest millimeter, at a magnification of 15 or 20. However, standard length was recorded if the margin of the posterior finfold was not clearly discernible. Conversion formulae were computed, based on 30 specimens from each of five *Sebastes* species (*alutus*, *babcocki*, *elongatus*, *reedi*, and *saxicola*) for which both total length and standard length were recorded. Size ranges of larvae ranged from 4.3 to 4.7 mm TL for *elongatus* to 6.1–6.5 mm TL for *alutus* (Fig. 1). Covariance analysis of species regression parameters indicated that at $P = .05$, only the intercepts for the SL-on-TL regressions were significantly different, and at $P = .01$, none of the four parameters differed significantly among species. Therefore, all-species regression formulae were computed for SL on TL and TL on SL which were:

$$SL = 0.979\ TL - 0.098$$

$$TL = 1.017\ SL + 0.124$$

Predicted values from these formulae were within

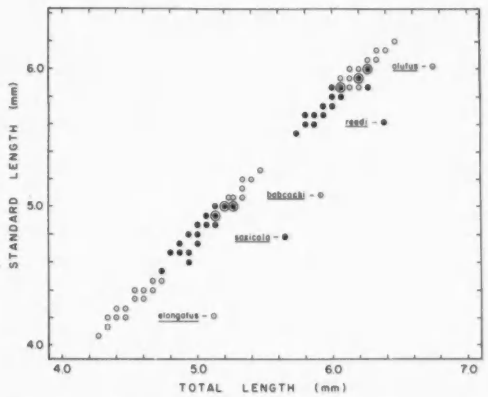

FIG. 1. Total length–standard length relationship for preextrusion larvae of five *Sebastes* species. (Intraspecific congruent points not indicated.)

± 4% of those provided by individual-species formulae.

Results

During 1963–73, a total of 66,760 specimens from 31 species of *Sebastes* was examined for gonad condition (Table 2). Collection during 1963–66 included only *alutus* (5,149 specimens), *jordani* (11 specimens), and *polyspinis* (63 specimens). Numbers sampled per species were roughly proportional to the incidence of the individual species in the on-bottom trawl catches of the *G. B. Reed*.

Inshore species, for which little effort was expended, included *caurinus*, *ciliatus*, *maliger*, *miniatus*, *nebulosus*, and *nigrocinctus*. Species uncommon to the study area were *goodei* and *jordani*, and those considered of limited availability to an on-bottom trawl were *entomelas*, *melanops*, *reedi*, *ruberrimus*, *variegatus*, and *wilsoni*.

Insemination

During September 1969, 1970, and 1972, male rockfish specimens were examined for gonad condition during other studies of Pacific ocean perch (*S. alutus*) off British Columbia. For most of the 18 species examined (including *S. alutus*), the gonads were in condition 8B (developing) (Table 3). For these species, the principal month of insemination was probably October. *Sebastes helvomaculatus* and *S. brevispinis* were the only species sampled in reasonable numbers, whose gonad development was relatively late, and in that case only in Queen Charlotte Sound (74 and 59%, respectively, condition 8A). Relatively early development was noted for *S. babcocki* (73% condition 8C) and *S. paucispinis* (84% condition 8C) in Queen Charlotte Sound, and *S. diploproa* (77% condition 8C) off west Vancouver Island. A possible source of error in determining time of insemination was the lack of spent (condition D) fish in sample, or worse, the failure to identify these. Lisovenko's (1970) description of *alutus* testes (Gulf of Alaska) suggests that the physical appearances of 8B and D testes were similar.

Bathymetric variation in testes development was noted for *S. alutus* sampled off southwest Vancouver Island during September 1970 and 1972. Testes development appeared to correlate with the "types" of Pacific ocean perch hypothesized by Westrheim (1973) — shallow-water and deepwater types. These were superficially delineated by the bimodal catch-rate histograms for

TABLE 2. Numbers of rockfish (*Sebastes*) specimens examined for gonad condition, by species, 1963–73.

Species	Number
aleutianus	1,977
alutus	27,256
aurora	95
babcocki	2,978
borealis	527
brevispinis	2,329
caurinus	45
ciliatus	22
crameri	2,288
diploproa	5,702
elongatus	4,623
entomelas	330
flavidus	905
goodei	1
helvomaculatus	5,027
jordani	12
maliger	119
melanops	15
miniatus	19
nebulosus	13
nigrocinctus	15
paucispinis	410
pinniger	412
polyspinis	63
proriger	4,076
reedi	1,063
ruberrimus	161
saxicola	253
variegatus	692
wilsoni	40
zacentrus	5,292
Total	66,760

TABLE 3. Distribution (percent) of mature male rockfish specimens, by gonad condition, collected off British Columbia, by area, September 1969–70 and 1972.

Species	Queen Charlotte Sound 1969			Numbers sampled	West Vancouver Island 1969–70, 1972			Numbers sampled
	8A	8B	8C		8A	8B	8C	
aleutianus	–	76	24	17	7	84	8	95
alutus	–	76	24	122	42	57	1	1,746
aurora	–	–	–	0	–	86	14	7
babcocki	3	24	73	70	–	50	50	2
borealis	–	–	–	0	3	97	–	39
brevispinis	59	41	–	63	50	50	–	6
crameri	–	67	33	3	21	64	15	66
diploproa	7	57	36	74	–	23	77	13
elongatus	11	78	11	9	4	75	21	24
entomelas	–	–	–	0	–	100	–	8
flavidus	–	37	63	59	–	50	50	2
helvomaculatus	74	26	–	89	6	94	–	47
paucispinis	–	16	84	32	–	50	50	2
pinniger	–	46	54	35	100	–	–	2
proriger	–	44	56	59	19	47	34	98
reedi	–	77	23	39	–	37	63	19
saxicola	–	–	–	0	100	–	–	1
zacentrus	2	37	62	63	–	–	–	0

1970 (Fig. 2) and 1972 (Fig. 3), and more explicitly by the age–length relationship of selected age-groups from the 1970 collection (Fig. 4). Interestingly, the testes-condition data suggest a later time for insemination for females in the deepwater stock (34–51% condition 8B) than for females in the shallow-water stock (84–95% condition 8B). If so, then the two stocks may be genetically separate. No other *Sebastes* species exhibited this phenomenon, perhaps because of a narrower depth range or inadequate sampling.

Bottom temperature might have affected testes development in the deeper water during 1970 — 6.0–6.3 C at 100–159 fath (183–291 m) vs. 5.1–5.2 C at 200–259 fath (366–474 m) – but in 1972, comparable temperatures were much higher (6.7–7.0 C at 100–139 fath (183–254 m), and 5.6–6.1 C at 200–239 fath (366–437 m)), and the differences in testes development were more pronounced.

Within the study region, other investigators have dealt only with Pacific ocean perch. Snytko (1970, 1971) and Gunderson (1971) reported insemination occurred during September–October in the B.C.–Oregon region. For the Gulf of Alaska, Lyubimova (1965) reported September–November, and Lisovenko (1970), October–January. In the Bering Sea, Paraketsov (1963) reported January–February, and Pautov (1972), September–October.

For southern California, Moser (1967b) reported insemination of *S. paucispinis* occurred during October–December. He further noted that testes development proceeded similarly for 30 other *Sebastes* species (not listed), and varied only in the time of initiation.

Parturition

Parturition season is at least January–July for *Sebastes* species in the study area (Table 4). Off British Columbia, two species (*paucispinis* and *pinniger*) gave birth to larvae prior to February, and six species (*aurora*, *brevispinis*, *diploproa*, *elongatus*, *proriger*, and *zacentrus*) later than June. For *alutus*, the principal month was March (Fig. 5). In the southeastern Gulf area, *alutus* and *babcocki* parturition occurred 1–2 months later (April–May) than off British Columbia. For *borealis* and *variegatus*, birth of larvae occurred in the same month as off British Columbia — April and June, respectively.

Bathymetric variation in ovary condition was noted in *S. alutus* samples collected off Vancouver Island during February 1969. Condition-4 fish constituted 69–73% at 160–199 fath (293–364 m), 40% at 200–219 fath (366–401 m), and 12–18% at 220–279 fath (402–510 m) (Fig. 6). Corresponding values for condition-5 fish were 18–28%, 59%, and 80–86%, respectively. The February 1967 sample was collected over a narrower depth range, and the March 1967 sample

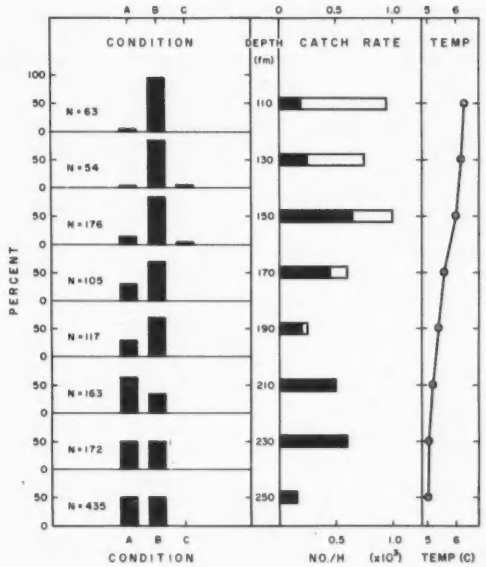

FIG. 2. Gonad condition and catch rate (number per hour) for male *S. alutus*, and bottom temperature (C, by 20-fath depth interval, off southwest Vancouver Island, September 1970. (Solid portion of catch-rate histogram indicates mature fish.)

contained virtually no condition-4 fish at any depth.

Since ovaries of the fish from deeper water were more advanced than those from shallower water, lower temperatures did not slow ovary development. Catch rates for mature fish were substantially lower in shallow water, and presumably if condition-5 fish were abundant there, they were less available to the on-bottom trawl used in this study. Westrheim (1973) noted the low catch rate of the shallow-water, fast-growing stock of *S. alutus* in this area during winter.

A coastwide review of parturition seasons for the *Sebastes* species in the study area reveals that parturition generally occurs earliest off California and latest in the Gulf of Alaska (Table 5).

For most *Sebastes* species, parturition occurs once annually, but some species exhibit two parturition seasons per year. Documentation of this has been reported for California species by Moser (1967a) (*paucispinis* and *goodei*), and MacGregor (1970) (*paucispinis*). Each author listed other species whose range did not extend north of California.

Off British Columbia, *S. diploproa* is the only species for which there is evidence of two parturition seasons per year. This evidence consists of substantial numbers of larvae-bearing females collected from two commercial trawl landings — one each in October and December 1973. The October landing was caught by midwater trawl off northwest Vancouver Island. The December landing was caught by on-bottom trawl in Queen Charlotte Sound. *Sebastes diploproa* may well release larvae throughout each year. Data collected during this study indicated condition-4 fish were present during all sampling months — February–July and September — in proportions varying from 2.0 to 52.9% of mature fish (Fig. 7).

Maturity

MATURATION

Three stages of maturation were identified for male and female specimens of all *Sebastes* species examined. These were immature, maturing, and mature. The maturing stage (condition 9 for males and condition 2 for females) has a minimum duration of 1 yr. Substantive documentation of this phenomenon was established for *S. alutus* collected off southwest Vancouver Island during 1967–72.

Size compositions of male and female *S. alutus*,

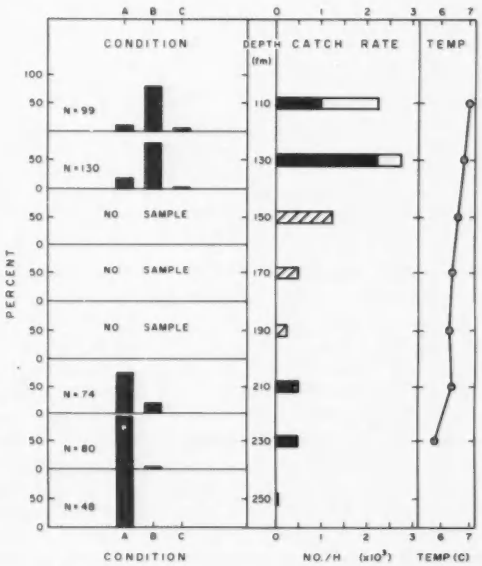

FIG. 3. Gonad condition and catch rate (number per hour) for male *S. alutus*, and bottom temperature (C), by 20-fath depth interval, off southwest Vancouver Island, September 1972. (Solid portion of catch-rate histogram indicates mature fish.)

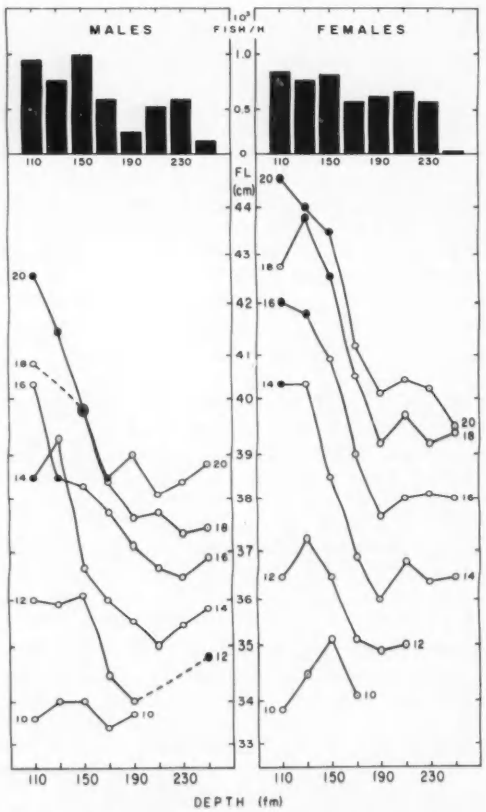

FIG. 4. Mean lengths (centimeters) of selected age-groups and catch rates (number per hour) for male and female *S. alutus*, by 20-fath depth interval, off southwest Vancouver Island, September 1972. (Solid dots indicate means based on less than five fish.)

by maturation stage, indicated the intermediate size of maturing fish, with respect to immature and mature fish, throughout the calendar year (Fig. 8). Mean lengths of maturing males were 33.6–34.8 cm, compared to 36.1–37.4 cm for mature fish. Mean length of maturing fish was smallest, and size distribution narrowest, at the onset of insemination (September), when mature males were most readily identified. Mean lengths of maturing females were 33.7–35.4 cm, compared to 38.1–39.0 cm for mature fish. Mean length of maturing fish was smallest at the onset of parturition (February), when mature females were most readily identified. The left skewness in the February size composition of maturing females may reflect the entrance of immature fish into the maturing stage, while the right skewness in September probably indicates the mature fish whose ovaries have not yet filled out to an identifiable "large" size (condition 3). Evidently, serious error can result in estimating size at maturity unless the maturing and mature stages can be readily separated. Optimum times for establishing length–maturity relationships differ among the sexes — at insemination for males and at parturition for females.

TABLE 4. Principal month of parturition for *Sebastes* species in the northeast Pacific Ocean, by area, 1963–70.

Species	Area: Southeast Gulf	Area: British Columbia
aleutianus	–	Apr.
alutus	Apr.–May	Mar.
aurora	–	> June
babcocki	May	Apr.
borealis	Apr.	Apr.
brevispinis	> May	> June
ciliatus	> May	–
crameri	–	Feb.
diploproa	–	July
elongatus	–	> June
entomelas	–	Apr.
flavidus	–	Mar.
helvomaculatus	> May	June
maliger	–	Apr.
melanops	–	< Apr.
nigrocinctus	–	May
paucispinis	–	< Feb.
pinniger	–	< Feb.
proriger	–	July
reedi	–	May
ruberrimus	–	May
saxicola	–	Feb.
variegatus	June	June
wilsoni	–	June
zacentrus	> May	July

SIZE (AGE) AT MATURITY

Length–maturity relationships were determined for 18 *Sebastes* species (Table 6). The size interval in which 50% of the fish are mature (hereafter called 50% maturity) ranged from < 17 cm for male *S. saxicola* to 48 cm for female *S. pinniger*. Females generally reached the 50% maturity level at a larger size than did males of the same species. Within species, size at 50% maturity generally varied inversely with latitude.

For Pacific ocean perch, males reached the 50% maturity level at 29 cm in the western Gulf of Alaska (February 1965 sample), 33 cm in the southeastern Gulf (March–May 1970), and 34 cm off Vancouver Island (September 1970 and 1972) (Fig. 9). Corresponding ages, based on formulae of Westrheim (1973, table 6), were 10,

TABLE 5. Principal month (or season) of parturition for selected *Sebastes* species of the northeast Pacific Ocean, by area.

Species	Area				
	Calif.	Oreg.–B.C.	SE Gulf[h]	E and W Gulf	Bering Sea
alutus	–	Jan.–Mar.[e,f,g,h]	Apr.–May	Apr.–June[m]	Feb.–May[n,o,p]
borealis	–	Apr.[h]	Apr.	–	–
brevispinis	–	> June[h]	> May	–	–
crameri	Nov.–Mar.[a]	Feb.[h]	–	–	–
diploproa	Feb.–July[a]	July[h,i]	–	–	–
flavidus	Nov.–Mar.[a]	Mar.[h]	–	–	–
helvomaculatus	–	June[h]	> May	–	–
jordani	Nov.–Mar.[d,c]	Mar.[j]	–	–	–
paucispinis	Oct.–Apr.[a,b,c,d]	< Feb.[h]	–	–	–
pinniger	Nov.–Mar.[a]	Jan.–Mar.[h,k,l]	–	–	–
ruberrimus	July–Aug.[b]	July[i]	–	–	–
saxicola	Nov.–Mar.[a]	Feb.[h]	–	–	–
variegatus	–	June[h]	June	–	–
zacentrus	–	July[h]	> May	–	–
melanops	–	Jan.[q]	–	Feb.[q]	–

[a]Phillips (1964); [b]Eigenmann (1893); [c]Morris (1956); [d]Moser (1967a); [e]Alverson and Westrheim (1961); [f]Snytko (1970); [g]Gunderson (1971); [h]This study; [i]DeLacy et al. (1964); [j]Westrheim and Pletcher (1966); [k]Fraser (1923); [l]Waldron (1968); [m]Lyubimova (1965); [n]Moiseev and Paraketsov (1961); [o]Skalkin (1964); [p]Pautov (1972); [q]Dunn and Hitz (1969).

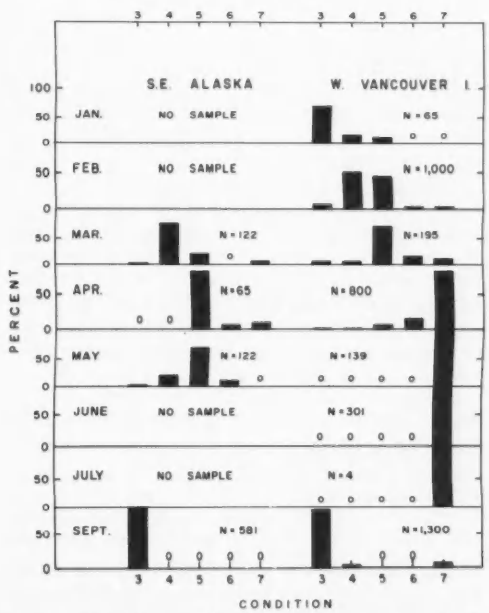

FIG. 5. Gonad condition, by month, for female *S. alutus* collected off southeastern Alaska and west Vancouver Island, 1967–73.

13, and 11 yr, respectively. Female Pacific ocean perch reached the 50% maturity level at 28 cm in the western Gulf (February 1965), 34 cm in the southeastern Gulf (March–May 1970), and 35 cm off Vancouver Island (February–March 1967 and 1969). Corresponding ages based on Westrheim's (1973) formulae were 10, 15, and 12 yr, respectively.

Snytko (1971) reported that Pacific ocean perch in the B.C.–Oregon region reached maturity at 32–35 cm (6 yr old). For the Gulf of Alaska (presumably including the southeastern Gulf), Lyubimova (1964) reported 20–28 cm (4–7 yr, Lyubimova 1965), and for the Bering Sea, Pautov (1972) reported that Pacific ocean perch reached maturity at 26–31 cm (6–9 yr old). The discrepancies in size at maturity between this study and the Soviet studies may have been caused by the inclusion of "maturing" fish with the mature.

Description of Larvae

Scorpaenid larvae are prominent members of the ichthyoplankton of the northeast Pacific Ocean (Aron 1960; Ahlstrom 1961; Lisovenko 1964; and Waldron 1972). Unfortunately, no identification key has been published to segregate the many species. East of 175°W, there are 69 species of *Sebastes*, two species of *Sebastolobus*, and one each of *Scorpaena* and *Scorpaenodes* (Chen 1971, 1975; Miller and Lea 1972; and Hart 1973). Only 36 *Sebastes* and two *Sebastolobus* species are found north of 42°N.

Intergeneric differentiation north of California (42°N) appears to be possible, based on size of larvae and melanophore pattern (Pearcy 1962).

TABLE 6. Length (centimeters) at 50% maturity for *Sebastes* species in the northeast Pacific Ocean, by sex and area.

Species	W Gulf		SE Gulf		W Vancouver Isl.	
	M	F	M	F	M	F
aleutianus	–	–	43	45	43	45
alutus	29	28	33	34	34	35
babcocki	–	–	–	–	38	42
borealis	–	–	34	37	45	45
brevispinis	–	–	–	–	43	45
crameri	–	–	–	–	34	37
diploproa	–	–	–	–	26	27
elongatus	–	–	–	–	23	23
entomelas	–	–	–	–	37	38
flavidus	–	–	–	–	40	42
helvomaculatus	–	–	21	21	22	20
pinniger	–	–	–	–	41	48
polyspinis	–	20[a]	–	–	–	–
proriger	–	–	–	–	28	29
reedi	–	–	–	–	37	38
saxicola	–	–	–	–	<17	<20
variegatus	–	–	22	23	24[b]	23[b]
zacentrus	–	–	23	25	24	25

[a]Westrheim and Tsuyuki (1971).
[b]Queen Charlotte Sound.

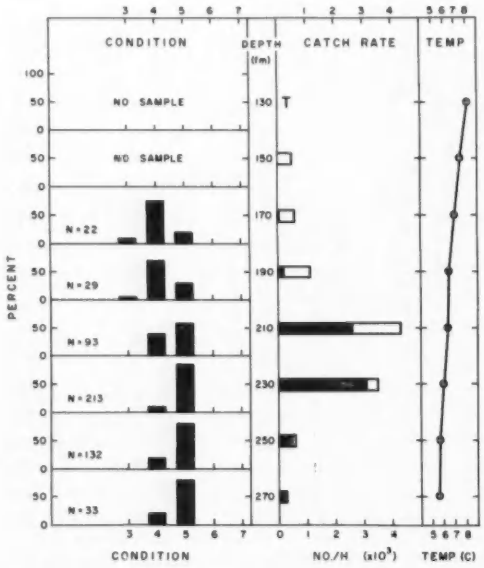

FIG. 6. Gonad condition and catch rate (number per hour) for female *S. alutus*, and bottom temperature (C), by 20-fath depth interval, off southwest Vancouver Island, February 1969. (Solid portion of catch-rate histogram indicates mature fish.)

Sebastes larvae are larger at birth than *Sebastolobus* larvae (4.0–7.2 mm vs. 2.6 mm). Aquarium-reared *Sebastolobus* larvae were only 3.5 mm TL 3 wk after hatching.

Interspecific differentiation based on pigment patterns, meristic counts, and/or morphometric ratios were reported by Morris (1956), DeLacy et al. (1964), Moser (1967b), and Efremenko and Lisovenko (1970). All authors expressed limited optimism for successful differentiation. In the present study, interspecific similarities and intraspecific differences have been discovered which indicate that morphological criteria alone will not be sufficient for interspecific differentiation of *Sebastes* larvae at parturition.

PREEXTRUSION LARVAE

During 1967–70, 993 preextrusion larvae were examined from 142 female *Sebastes*, comprising 16 species from three areas (Table 7). Interspecific similarities in pigment patterns were noted for *helvomaculatus-ruberrimus*, *babcocki-zacentrus*, and *proriger-zacentrus*. Intraspecific differences were noted for virtually all species collected from two or more areas, even those as close as Queen Charlotte Sound and southwest Vancouver Island. Furthermore, variation was sometimes noted among larvae from the same female. Thus, the evident variability of preextrusion larvae,

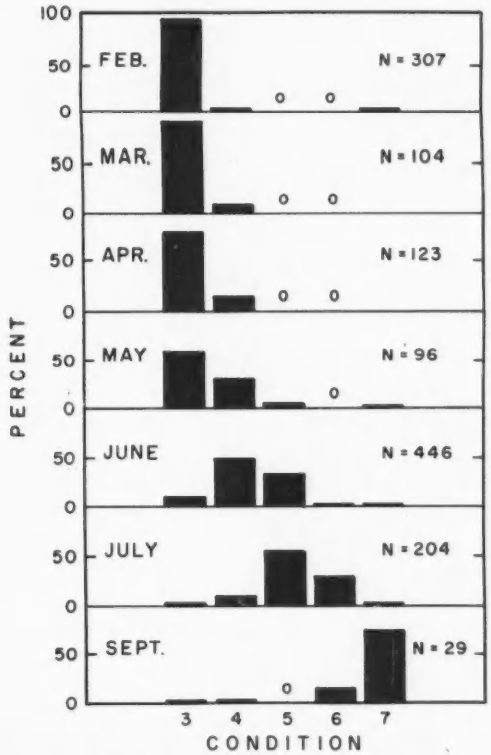

FIG. 7. Gonad condition of female *S. diploproa* collected off British Columbia, by month, February–September 1967–73. (July sample from Queen Charlotte Sound. All others off southwest Vancouver Island.)

based on just 16 *Sebastes* species, precludes successful species differentiation based only on conventional morphological criteria.

POSTEXTRUSION LARVAE

Analysis was completed on 39 lots comprising 13 species of *Sebastes* larvae reared aboard the *G. B. Reed*. Holding period ranged from 3 to 13 days per lot. No change in melanophore pattern was noted for *aleutianus* or *helvomaculatus* (southeast Gulf only).

Change in the ventral pigment row was noted for *elongatus* and *entomelas*. For both, the melanophore count increased to more than 16.

Changes in the dorsal pigment row were noted for *alutus*, *proriger*, and *variegatus*. The dorsal pigment row was present in 60% of the *alutus* lots at 4–8 days, and in 50% of the *variegatus* lots at 7 days. Both lots of *variegatus* possessed dorsal pigment rows at 3–4 days.

For 11 species, pigment spots appeared on the head, nape, and/or lower jaw during the holding period. These were *babcocki*, *ciliatus*, *crameri*, *elongatus*, *helvomaculatus* (southwest Vancouver Island only), *maliger*, *proriger*, *ruberrimus*, *variegatus*, and *zacentrus*.

One species, *ruberrimus*, acquired a pigment spot in the hypural region during its 7-day holding period.

Discussion

This limited study has yielded some interesting facts of *Sebastes* biology. Four of some importance are: 1) the bathymetric variation in development of *S. alutus* testes; 2) the intermediate, maturing stage in development of *Sebastes* gonads; 3) latitudinal variation in size (age) at maturity for *S. alutus*; and 4) the interspecific similarities and intraspecific differences in *Sebastes* larvae.

Bathymetric variation in testes development of *S. alutus* specimens collected off southwest Vancouver Island indicates the possibility of genetic isolation for the shallow-water and deepwater types, delineated by the age–length relationship (Westrheim 1973). Rehabilitation of the depleted shallow-water portion of the *S. alutus* stock (Westrheim et al. 1972) off Vancouver Island

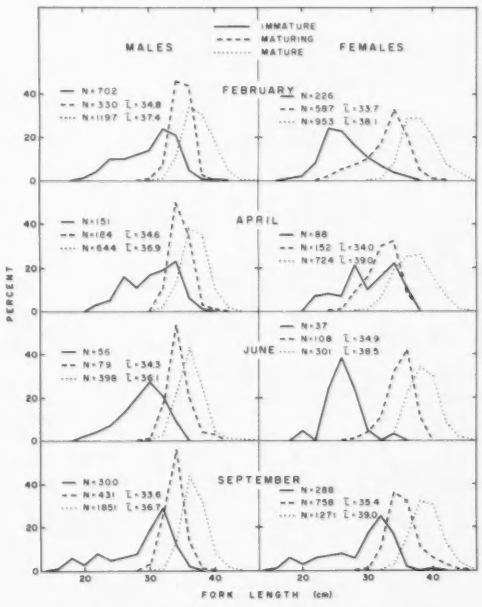

FIG. 8. Size composition, by sex by selected month, of immature, maturing, and mature *S. alutus* collected off British Columbia, February–September 1967–73.

TABLE 7. Pigment patterns of preextrusion larvae from 24 *Sebastes* species (Numerals are %; + = 100%, − = 0%).

	aleutianus	alutus		auriculatus	babcocki		brevispinis	caurinus	ciliatus	crameri	diploproa		elongatus	entomelas	flavidus	goodei	helvomaculatus	jordani	maliger		paucispinis	pinniger	proriger	reedi	ruberrimus		saxicola		variegatus	zacentrus
Source[a]	1	1	2	2	1	2	2	2	1	1	1	2	1	1	2	3	1	3	1	2	3	4	1	1	1	2	1	3	1	1
Character																														
Ventral pigment row																														
present	98	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
stops short of anus	98	+	+	—	+	+	+	—	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+	+	+	+	90	—	+	+
multiple or irregular	21	—	+	+	90	+	—	+	—	—	—	—	—	—	—	?	—	?	76	+	?	+	—	—	—	+	25	?	—	—
stops short of anus by as much as 4 myotomes	89	94	—	—	99+	—	—	—	+	74	98	+	+	+	+	+	82	+	—	—	+	+	94	5	33	—	65	—	+	99
usually < 16 melanophores	95	28	—	—	2	—	—	—	—	45	87	—	80	80	+	—	83	—	—	—	+	+	31	+	+	—	—	—	16	21
Dorsal pigment row																														
present	—	9	—	+	+	+	—	+	40	—	—	—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	+	+	—	—
stops short of anus	—	9	—	+	+	+	—	—	40	—	—	—	—	—	—	—	—	+	69	+	—	—	—	—	—	—	+	+	—	—
multiple or irregular	—	—	—	+	+	+	—	+	—	—	—	—	—	—	—	—	—	?	21	—	—	—	—	—	—	—	—	?	—	—
Head																														
or nape with some pigment	—	1	—	+	—	+	—	+	—	—	—	+	10	—	—	+	—	—	59	+	—	—	13	—	—	—	3	—	—	—
with 2-5 melanophores	—	1	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	21	—	—	—	—	—	—	—	—	—	—	—
with >5 melanophores	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	—	—	—
lower jaw with some pigment	—	T	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14	—	—	—	—	—	—	—	—	—	—	—
Hypural region																														
with pigment spot(s)	93	—	—	—	96	+	+	—	90	89	83	+	—	—	+	—	1	—	—	—	—	—	64	27	—	+	—	—	83	71
Mean total length (mm)	4.9	6.3	5.3	5.8	5.4	5.0	4.0	5.5	5.8	5.7	4.4	5.0	4.8	5.0	4.5	5.7	4.1	6.8	5.5	5.0	6.0	—	4.9	5.8	4.3	5.0	5.0	4.3	4.7	4.3
No. of larvae	57	355	4	?	100	4	?	?	10	60	60	4	20	5	?	?	120	?	29	4	?	—	85	63	15	4	40	?	35	70
No. of females	7	52	1	?	9	1	?	?	2	7	6	1	3	1	?	?	17	?	4	1	?	—	12	7	2	1	4	?	7	8

[a]1, This study; 2, DeLacy et al. (1964); 3, Morris (1956); 4, Waldron (1972).

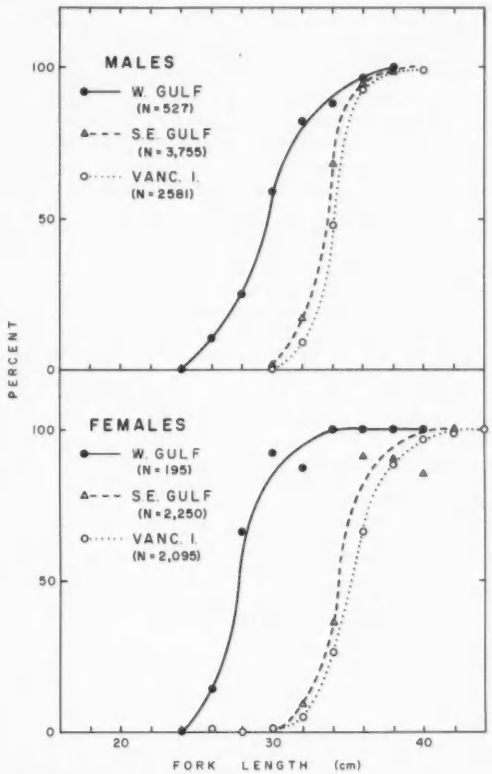

FIG. 9. Length–maturity relationship for male and female *S. alutus* collected in the northeast Pacific Ocean, by area, 1963–73.

may be more difficult if the deepwater portion of the stock is indeed genetically independent of the shallow-water portion.

Presence throughout the year of the intermediate, maturing stage of *Sebastes* gonads provides the opportunity for the unwary to underestimate the true size (age) at maturity for both males and females. This source of error will be minimized if size (age) at maturity is established from samples collected at the peak development stages — insemination season for males, and parturition season for females.

Latitudinal variation in size (age) at maturity observed in this study corroborates, in general, the results of Lyubimova (1964, 1965) and Snytko (1971). This variation is additional evidence for the existence of discrete stocks of Pacific ocean perch in the north Pacific Ocean. Interestingly, the length–maturity relationship of this study suggests that the southeast Gulf fish are more similar to British Columbia fish than west Gulf fish, while size composition (Westrheim 1970. fig. 8) and age–length (Westrheim 1973, fig. 7) data suggest the opposite.

Interspecies similarities and intraspecies differences in morphometric and meristic characteristics of preextrusion *Sebastes* larvae delineated in this study clearly preclude accurate identification, based only on these criteria, of *Sebastes* larvae caught at sea. Perhaps the most feasible technique at this time will be deducing the probable identification of such larvae based on parturition season and relative abundance of adult members of likely *Sebastes*.

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D. R. Gunderson and K. S. Ketchen reviewed the manuscript, and provided a number of constructive suggestions.

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Phytoplankton Succession and Primary Production in Babine Lake, British Columbia

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STOCKNER, J. G., AND K. R. S. SHORTREED. 1975. Phytoplankton succession and primary production in Babine Lake, British Columbia. J. Fish. Res. Board Can. 32: 2413–2427.

Ten stations located in six zones (subbasins) were sampled biweekly from May to October 1973 to detect possible regional differences in production in this large, 155 km long, dystrophic lake. The spring bloom occurred in all zones while a fall bloom occurred only in Zones 5 and 6. Carbon assimilation showed two peaks in south basin zones, but only one (spring) at zones north of Topley Landing. Seasonal variation in phytoplankton numbers and volume, seston, and chlorophyll *a* followed a pattern similar to that noted for primary production. Mean production was 100 mg $C \cdot m^{-2} \cdot day^{-1}$ in Zones 1–4, but was 145 in Zones 5 and 6. Annual production was estimated at 25 g $C \cdot m^{-2}$ in the north basin and 40 in the south basin. Reasons for the regional disparities are discussed, with greatest significance given to regional variations in mixed layer depth, surface inflows (loading), and basin mean depth. The development and sustainment of the autumnal bloom of *Tabellaria fenestrata* is thought to be one of the principal factors responsible for greater production in the south basin.

An estimated 0.05 g $TP \cdot m^{-2}$ enters the lake yearly. This can vary depending on the return of adult sockeye salmon (*Oncorhynchus nerka*), whose carcasses contribute up to 20% of the total. An estimated 30% is lost via the Babine River, and it is speculated that of the remaining 70%, most is lost to the sediments. Phosphate limitation is implied as a chief factor limiting primary production in the north basin stations, but not in the south basin. On the basis of total phosphorus load the lake is classed as oligotrophic, but in terms of annual production and its humic stained waters it is more correctly considered mixotrophic.

STOCKNER, J. G., AND K. R. S. SHORTREED. 1975. Phytoplankton succession and primary production in Babine Lake, British Columbia. J. Fish. Res. Board Can. 32: 2413–2427.

Dix stations situées dans six zones (sous-bassins) ont été échantillonnées deux fois par semaine de mai à octobre 1973 en vue de détecter des différences régionales possibles de production dans ce grand lac dystrophe de 155 km de longueur. La poussée planctonique vernale se produit à la plupart des stations tandis que la poussée automnale ne se produit qu'aux zones 5 et 6. L'assimilation du carbone montre deux pics aux stations du bassin du sud, mais un seul (au printemps) aux stations situées au nord de Topley Landing. La variation saisonnière du nombre et du volume de phytoplancton, du seston et de la chlorophylle *a* suit le même schéma que celui observé pour la production primaire. La production moyenne aux zones 1–4 est de 100 mg $C \cdot m^{-2} \cdot jour^{-1}$ mais elle est de 145 aux zones 5 et 6. On estime à 25 g $C \cdot m^{-2}$ la production annuelle dans le bassin du nord et à 40 dans le bassin du sud. Nous examinons les facteurs responsables des disparités régionales, avec accent sur les variations régionales à la profondeur de la couche mixte, aux apports de surface (charge) et à la profondeur moyenne des bassins. On est d'avis que le développement et le maintien de la poussée automnale de *Tabellaria fenestrata* est un des principaux facteurs responsables d'une production plus grande dans le bassin du sud.

Nous avons calculé la charge de phosphore et estimé à 0.05 g $PT \cdot m^{-2}$ la quantité qui pénètre annuellement dans le lac. Ce taux peut varier en fonction du nombre de saumons nerka (*Oncorhynchus nerka*) adultes qui retournent au lac et dont les carcasses contribuent jusqu'à 20% de la charge annuelle. Environ 30% du phosphore total qui entre dans le lac en sort par

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l'émissaire, la rivière Babine, et on suppose que la balance de 70% se perd dans les sédiments. On est porté à croire que la limitation des phosphates est le principal facteur limitant la production primaire dans les stations du bassin du nord, mais non dans le bassin du sud. Sur base de charge de *phosphore total, ce lac entre dans la catégorie des oligotrophes, mais en termes de production* annuelle et d'eaux humiques teintées, nous le considérons plus correctement comme mixotrophe.

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MAJOR industries have been active on the Babine Lake watershed for several years and their activities are expected to increase in the future. Baseline studies were urgently needed to assess future changes in water quality, since water quality control is vital to the perpetuation of the salmon enhancement program initiated in 1965. Spawning channels were constructed on Fulton River and Pinkut Creek to increase sockeye salmon (*Oncorhynchus nerka*) fry production in the lake. In 1973 numbers of underyearling sockeye in Babine Lake were estimated to be 3–4 times higher than in preenhancement years.

To assess effects of increased underyearling sockeye populations on lake production, programs were initiated to investigate both primary and secondary production. The need for productivity work was emphasized by the fact that studies that initially suggested that Babine Lake was underutilized as a nursery area for underyearling sockeye salmon were based on a "uniform distribution hypothesis" (Johnson 1958). It has since been found that the distribution of underyearlings is not uniform (McDonald 1969), and so previous estimates of Babine Lake carrying capacity may be incorrect. The present paper reports results of primary production, phytoplankton succession, and nutrient loading studies carried out in 1973–74.

Description of Study Area

GEOLOGICAL SETTING

The major structural trend of the area around Babine Lake, as indicated by strikes of dipping beds and major faults, is northwesterly. Northeasterly trending faults, while present, are of lesser regional importance. In the northern sectors, scattered and sometimes economic copper deposits are locally associated with Plutonic rocks. Pleistocene glacial and postglacial tills and drift now cover much of the area.

MORPHOMETRIC AND PHYSIOGRAPHIC FEATURES

Babine Lake lies 580 km north of Vancouver on the Central Interior Plateau of B.C. (lat. 55°N, long 123°W) (Fig. 1). It is 155 km long, 9 km wide at the widest point, and has an area of 490 km^2. The main lake has a mean depth of over 70 m, the North Arm and Morrison Arm less than 20 m, and Hagan Arm less than 30 m (Table 1). Mean annual discharge from Babine Lake is about 47 $m^3 \cdot s^{-1}$, with peak flows of 110 in June. Theoretical water renewal rate (flushing) is about 20 yr.

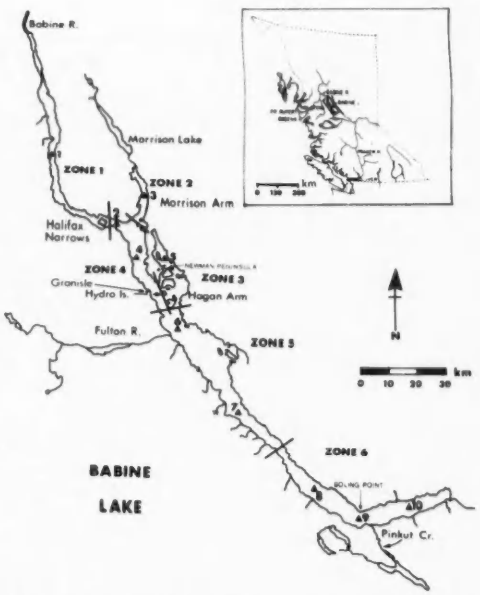

FIG. 1. Babine Lake showing station locations and salient geographical information.

The watershed surrounding Babine Lake totals about 10,000 km^2 and forms one of the major drainage basins of the Skeena River system. Precipitation is distributed fairly evenly throughout the year, and including 2.5–3.0 m of snow, is about 60 $cm \cdot yr^{-1}$. The lake is usually ice covered from December to early May. Summer air temperatures range from 8 to 27 C with a mean of around 16 C. Winter temperature may plunge to *−40 C for long periods, but average only −16 C* for winter months.

RECENT HISTORY

The area aroud Babine remained thinly populated (ca 300) until the early 1960s. The town-

TABLE 1. Babine Lake morphometry by zone.

Zone	A[a] (km^2)	Vol ($m^3 \times 10^6$)	$\bar{Z}$ Mean depth (m)	Zm Max. depth (m)	L Shoreline (km)	D_L	l length (km)	$\bar{X}$ Annual discharge ($m^3 \cdot s^{-1}$)	Theoretical resident time (yr)
1	53.40	1,076	18.7	46	–	–	39.8		
2	17.72	202	11.4	31	–	–	13.4		
3[b]	49.25	931	25.0	83	–	–	9.0		
4[b]	73.32	5,090	62.8	144	–	–	25.5		
5[b]	164.26	8,065	51.9	141	–	–	39.5		
6	132.76	11,646	87.5	186	–	–	45.5		
Lake total	490.71	27,010	55.1	186	364.7	7.19	150	47.01[c]	18.2

[a]After Johnson (1965b).
[b]Extrapolated from Johnson (1965b).
[c]From 1945 to 1970 (Water Survey Data).

site of Granisle was developed in 1962–63 and currently accommodates about 2000 people primarily associated with the mining industry. During the ice-free months the population increases considerably with an influx of tourists, hunters, and various seasonal workers associated with the mining, fishing, and forest industries.

RESOURCE UTILIZATION

The Babine watershed contains extensive high-yield forests, primarily composed of white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta v. latifolia*). Small-scale logging occurred in the area as early as 1925, but was selective, and did little apparent environmental damage. Clear-cut logging began in the 1950s, and by the late 1960s resulted in several openings in excess of 200 ha. Currently two copper mines conduct extensive open-pit operations on Copper Island in Hagan Arm and on Newman Peninsula in the north basin, main lake (Fig. 1).

Babine Lake supports large populations of rainbow trout (*Salmo gairdneri*), kokanee (landlocked sockeye, *Oncorhynchus nerka*), lake trout (char, *Salvelinus namaycush*), and lake whitefish (*Coregonus clupeaformis*). Sockeye salmon are the primary commercial species in the Babine system.

Methods

FIELD PROCEDURE

Primary production — With minor modifications, the standard ^{14}C method as initially proposed by Steeman-Neilsen (1952) was used. Water was collected from eight depths (0, 1, 2, 3, 5, 10, 20, and 30 m) in a 6-liter plastic Van Dorn bottle. Of 10 stations regularly sampled, two were at depths less than 30 m. At these stations water was collected to 20 m only.

Two 125-ml light bottles and one 125-ml dark bottle were filled from each depth and inoculated with approximately 3 μCi of $NaH^{14}CO_3$ radio-isotope stock (New England Nuclear N.E.N.). In all production sets from May to July, productivity bottles were inoculated with one ampoule (1 ml) of radio-isotope stock. From August to October, to reduce ampoule-to-ampoule variation, ampoules were mixed prior to inoculation and diluted with an equal portion of distilled water. Productivity bottles were then inoculated with 1 ml of this ^{14}C stock solution. To obtain a DPM count for each batch of diluted radio-isotope stock, three scintillation vials were inoculated with 1 ml each of the stock solution.

After inoculation of samples, bottles were suspended from clear plexiglass holders and incubated for 4 h at the original sampling depths. After retrieval, bottles were stored in a light-tight container and transported to the laboratory for immediate filtration. Time between retrieval and filtration never exceeded 3h.

Chlorophyl a and seston — One liter for both chlorophyll *a* and seston was collected in plastic bottles from each of the eight depths listed above. These were transported to the laboratory for immediate filtration and storage prior to analysis.

Phytoplankton standing crop — Water samples (100 ml) were collected in plastic bottles from each of the eight depths. These were preserved with Lugol's acetate solution and used for direct counts of phytoplankton.

Physical parameters — Belfort recording pyroheliometers were placed at three locations, in the study area: Halifax Narrows, Hydro Island, and Pinkut Creek (Fig. 1). For every station sampled the total record of the closest pyroheliometer for that date was planimetered. The record for the incubation period was also planimetered and converted to a percentage of the entire light day. These data were then incorporated into the productivity equation of Strickland (1960). A Montedoro-Whitney illuminance meter (Model LMT-8B) was available for a portion of the study and was used to measure light extinction as a function of depth. These data were plotted on a Hewlett-Packard Calculator-Plotter as

the natural log of intensity vs. depth. A line was regressed through the points and its slope gave mean extinction coefficient. A standard 22-cm white Secchi disc was used to measure water transparency at every set.

A bathythermograph was lowered at each station to obtain a temperature–depth profile, and surface temperature for BT calibration was measured with a standard bucket thermometer.

Chemical parameters were monitored at five stations in May, August, and October, 1973. Analyses were conducted at the Fisheries Analytical Laboratory, Pacific Environment Institute, West Vancouver. Methods used are described in Fisheries/EPS Chemistry Laboratory Manual (1974).

Laboratory Procedure

All primary production, chlorophyll *a*, and seston samples were filtered at 20 cm Hg on the same day they were taken.

Primary production — Productivity bottles were filtered onto 0.45-μ pore size, 25-mm diam, cellulose acetate Sartorius membrane filters. After filtration, filters were immediately placed in scintillation vials containing 10 ml of Aquasol (N.E.N., L.S.C. fluor). Scintillation vials were shipped by air to the Vancouver Laboratory of the Fisheries and Marine Service for counting.

The activity of samples was determined on a Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3375). The equation of Strickland (1960) was used to convert counts per minute to milligrams $C \cdot m^{-3} \cdot day^{-1}$.

Profiles of milligrams $C \cdot m^{-3} \cdot day^{-1}$ as a function of depth were integrated by computer to give production on an areal basis (milligrams $C \cdot m^{-2} \cdot day^{-1}$). To obtain estimates of annual production, values were plotted and planimetered over a 275-day period, representing the period from the onset of production under ice in April to the decline to negligible values in November.

Chlorophyll a — Chlorophyll samples were filtered onto 5.5-cm diam, Whatman GF/C glass fiber filters. Two milliliters of saturated $MgCO_3$ solution were added to each sample to prevent acidification prior to analysis. Filters were folded, placed in aluminum weighing dishes, and frozen.

During analysis of samples it was found that there was little variation in chlorophyll values in the upper depths, (0–5 m) and thus only samples from 1, 3, 5, and 20 m were analyzed. Analysis was according to methods of Strickland and Parsons (1972), with some samples analyzed using methods of Yentsch and Menzel (1963). There was good agreement between methods.

Seston — Seston samples were filtered, ashed, and weighed using 5.5 cm Whatman glass fiber filters. Filters were folded, placed in aluminum weighing dishes, and frozen.

Seston analysis was carried out at the Fisheries and Marine Service Laboratories, Pacific Environment Institute. Samples were dried at 105 C to constant weight, weighed, ashed in a muffle furnace at 500 C for a minimum of 4 h, and weighed again for loss on ignition.

Phytoplankton standing crop — Phytoplankton were counted using the Utermöhl (1958) settling method on a Zeiss inverted plankton microscope. In preparation for counting, a sample bottle was thoroughly shaken, and a portion used to completely fill a 25-cm^3 settling chamber. Plankton was allowed to settle overnight before counting.

Two complete transects at 160× and 400× magnification were counted; however, only those at depths above the thermocline were included in subsequent calculations. When light data were available (after September 1), only those samples enumerated at or above the compensation depth (1% of surface light) were included in calculations. In actual practice, both methods resulted in excluding samples from 20 to 30 m from any calculations. However, under isothermal conditions in May and early June, samples from all eight depths were enumerated.

Counts were converted to number of cells $\cdot m^{-3}$ and to total phytoplankton volumes ($mm^3 \cdot m^{-3}$). Phytoplankton volumes were determined using values calculated by Evans and Stockner (1972) and by Nauwerck (1963), although direct volume estimates for certain species were made by the authors.

Rationale for Station Locations and Sampling Strategy

From the outset it was considered essential to sample the major basins of Babine Lake at sufficient intervals to indicate spatial and temporal changes in phytoplankton species succession. The large distances to be covered on a lake of this size, as well as manpower availability, determined that 10 stations were the maximum that could be sampled at biweekly intervals.

Data interpretation for presentation of results was facilitated by dividing the lake into six zones, each with one or more stations (Fig. 1). Boundaries were based chiefly on known morphometric, physical, and biological differences among regions (Johnson 1956, 1958; MacDonald and Scarsbrook 1969).

Results

Physical Parameters

Light — Mean extinction coefficients, (number of observations in parentheses) for the fall period in each zone were: 1) .577(2); 2) .669(3); 3) .593(2); 4) .593(4); 5) .598(4); 6) .771(6). A gradual increase in values from north to south basins is apparent (0.578–0.771).

Secchi depth — Zone 2 had the lowest mean Secchi depth and Zone 4 the highest (Table 2). Highest Secchi values were coincident with low phytoplankton standing crops, and low values occurred during the vernal bloom at all stations and autumnal bloom at southern stations.

TABLE 2. Seasonal average values by zone of selected parameters: Secchi depth, seston, chlorophyll *a*, phytoplankton volume, and annual areal production.

Zone (stations)	$\bar{x}$ Secchi depth (m)	$\bar{x}$ Dry seston ($g \cdot m^{-3}$)	Chlorophyll *a* ($\bar{x}$ $mg\ chl\ a \cdot m^{-3}$)	$\bar{x}$ Phytoplankton vol ($mm^3 \cdot m^{-3}$)	Production ($g\ C \cdot m^{-2} \cdot 275\ days^{-1}$)
1 (1)	4.5	1.74	1.6	159	23.2
2 (3)	4.3	1.47	2.0	105	29.7
3 (5)	5.8	1.35	1.5	175	29.3
4 (2,4)	5.9	1.21	1.4	164	27.7
5 (6,7)	5.4	1.63	2.1	373	36.8
6 (8,9,10)	5.5	1.57	2.1	381	42.0

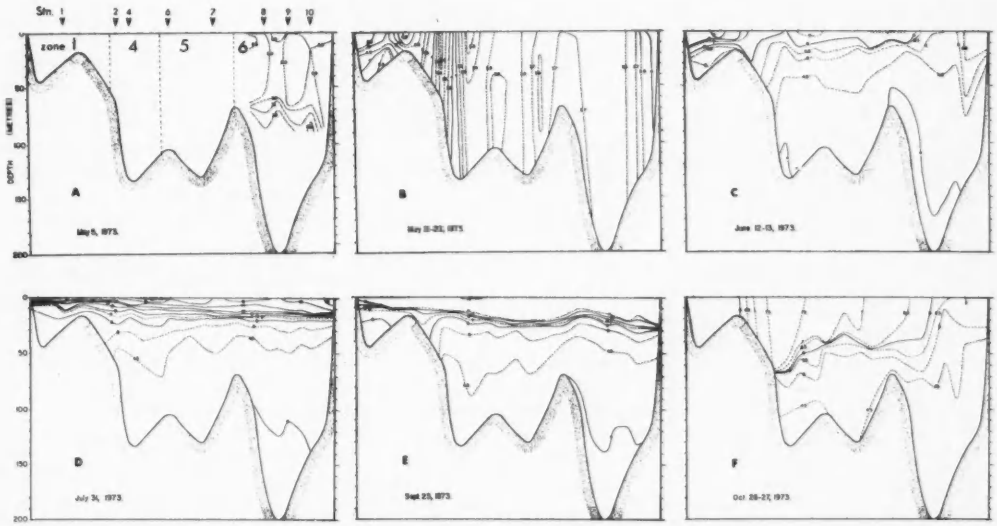

FIG. 2. Temperature isotherms on selected dates in 1973, Babine Lake (D. Farmer unpublished data).

Temperature — Babine Lake is dimictic. In 1973 cool temperatures and cloudy conditions delayed the onset of thermal stratification until late June (Fig. 2). Autumnal winds and cool temperatures in October restored isothermal conditions which remained to the end of November and commencement of ice cover (Fig. 2). The daily variation of mixed layer depth was monitored by D. Farmer (unpublished data) and permitted recognition of zones of upwelling, intense mixing, and relative stability among various regions (basins) of the lake.

Chemical parameters — Phosphate and nitrate concentrations showed little spatial or temporal variation (Stockner and Shortreed 1974). This fact may be more related to insufficient sensitivity of analytical methods than to actual observation. In June and July silicate concentrations were similar throughout the lake, but by late September were considerably lower in the south basin (Zone 6). Total alkalinity showed little spatial variation in 1973, with the exception of Zone 2, where values were lower (Table 3). There was some zonal variation in conductivity, with highest values occurring in the north basin in the fall. Results of chemical studies reported by Narver (1967), Stephens et al. (1969), and Smith and Davidson (unpublished data) showed similar temporal and spatial constancy in parameters analyzed.

Babine waters are noticeably stained by humic substances. Average color in Hazen units varied from 10 to 40, with North, Hagan, and Morrison arms the most stained.

TABLE 3. Mean concentration of selected chemical parameters in 1973 (Smith and Davidson unpublished data).

Zone	TPO_4[a] ($g \cdot m^{-3}$)	NO_2-NO_3 ($g \cdot m^{-3}$)	SiO_2 ($g \cdot m^{-3}$)	Cond.	Color (*Hazen units*)	T. Alk ($g \cdot m^{-3} CaCO_3$)
1	<.02	.07	4.3	119.5	13	–
2	<.02	.06	3.3	84.3	13	30.8
4	<.02	.07	3.3	82.1	13	36.2
5	<.02	.06	3.2	84.6	15	36.7
6	<.02	.06	3.1	84.6	11	36.4

[a]Analysis in 1974 by B.C. Water Resources Laboratory shows mean lake values of $PO_4(P)$ of <0.003 and TP of 0.007.

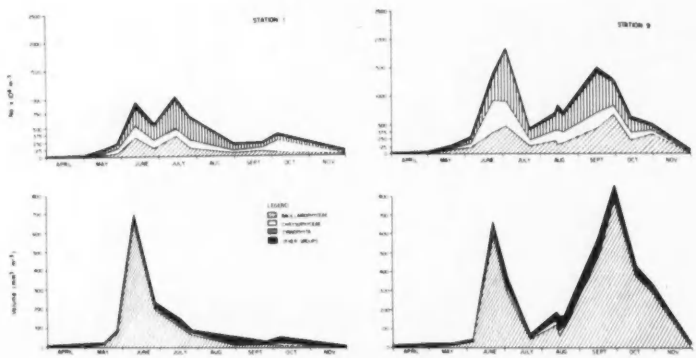

FIG. 3. Seasonal variation of phytoplankton numbers and volume at Stations 1 and 9, Babine Lake.

BIOLOGICAL PARAMETERS

Seston — Highest mean seston occurred in Zone 1 (Table 2). Of the main lake, the northern basin (Zone 4) had a somewhat lower mean seston than did the southern (Zones 5 and 6).

Prior to the onset of stratification, seston was uniformly distributed in the water column, but by July greatest concentrations occurred between 1 and 5 m, with sparse amounts noted in the 0–1-m layer and at depths below 5 m. In September and early October in the south basin greatest seston occurred in the surface layer (0–1 m).

Chlorophyll a — Highest mean values occurred in Zone 6 in the south basin (Table 2). Mean concentrations in the north basin of the main lake (Zone 4) were considerably lower. Vertical distribution of chlorophyll *a* was similar to that noted for seston.

PHYTOPLANKTON

Species composition — Diatoms, cyanophytes, and chrysophyceans were the major groups found in Babine Lake in 1973 (Table 4).

Common diatoms in the lake were *Rhizosolenia longiseta*, *Cyclotella stelligera*, *C. ocellata*, *Tabellaria fenestrata*, *Fragilaria crotonensis*, *F. construens*, *Melosira italica*, and *Asterionella formosa*. *Oscillatoria* sp. and *Chroococcus* sp. were the dominant blue-greens, although at times *Anabaena* occurred in considerable numbers. Among chrysophyceans, *Chromulina* sp. and *Chrysochromulina* sp. were most important, with *Mallomonas* spp. common in most zones.

Numbers — In early May, all groups were at low levels and in about equal proportions throughout the lake. The spring bloom in all areas was caused by a substantial increase in numbers of all groups (Fig. 3). Zones 5 and 6 (Stations 6–10) in the south basin had considerably more phytoplankton in spring and also a considerable fall bloom, lacking in Zones 1–4 (Stations 1–5).

Volume — Plots of seasonal variation in phytoplankton volume are considerably different than numerical plots, chiefly because of the large size of common diatoms and the small size of common blue-greens (Fig. 3). In all zones peak

TABLE 4. Major algal species in Babine Lake and their respective volume estimates.

BACCILARIOPHYCEAE	
Dominant genera	Volume (μ^3)
Asterionella formosa (Hantz) Grun.	100
Cyclotella stelligera Cleve & Grun.	30
Fragilaria crotonensis (Kitton)	120
Melosira italica (Ehr.) Kutz	90
Rhizosolenia longiseta Zach.	3,000
Tabellaria fenestrata (Lyngb) Kutz	3,000
Others	
Achnanthes spp.	30
Cocconeis spp.	360
Cymbella spp.	340
Epithemia spp.	300
Gomphonema spp.	330
Navicula spp.	90
Nitzschia spp.	180
Rhopalodia sp.	4,000
Stephanodiscus astrea (Ehr.) Grun.	60
Synedra spp.	770
CHRYSOPHYCEAE	
Dominant genera	Volume (μ^3)
Chromulina sp.	50
Chrysochromulina sp.	50
Others	
Dinobryon sp.	350
Mallomonas sp.	250
CYANOPHYCEAE	
Dominant genera	Volume (μ^3)
Chroococcus sp.	4
Oscillatoria sp.	3
Others	
Anabaena sp.	20
Gleocapsa sp.	4
Merismopedia sp.	5
DINOPHYCEAE, CHLOROPHYCEAE, CRYPTOPHYCEAE	
Dominant genera	Volume (μ^3)
Ankistrodesmus sp.	250
Ceratium sp.	10 000
Others	
Closterium sp.	4 000
Cryptomonas sp.	2,500
Gymnodinium sp.	400
Quadrigula sp.	250
Staurastrum sp.	20,000

values in spring occurred when *Rhizosolenia longiseta* was at its population maximum. Fall increases in the south basin were caused primarily by increases of *Tabellaria fenestrata* and *Fragilaria* spp. An increase of "other groups," apparent in all areas immediately after the spring bloom and again in the fall, was caused mainly by *Cryptomonas* sp. and *Ankistrodesmus* sp.

Zones 5 and 6 had the highest phytoplankton concentrations while Zones 1–4 had considerably lower concentrations (Table 2). A more detailed discussion of seasonal phytoplankton succession in Babine Lake appears in Stockner and Shortreed (1974).

Primary production — Temporal variation of areal rates — Production in northern zones (1–4) showed only a vernal peak while Zones 5–6 in the south basin showed considerable vernal as well as autumnal increases (Fig. 4). These differences are reflected in annual rates, where production ranged from a low of 23.2 g $C \cdot m^{-2}$ in Zone 1 to a high of 42.0 in Zone 6 (Table 2). The mean annual production in the north (mean Zones 1–4) was 27 g $C \cdot m^{-2}$, contrasting to a mean of 40 (Zones 5 and 6) in the south. The mean of all zones was 31.5 g $C \cdot m^{-2}$.

Vertical production profiles — There was a basic similarity in vertical profiles of carbon assimilation among zones and stations (Stockner and Shortreed 1974). The most notable feature was that between 80 and 90% of carbon assimilation occurred within the 0–5-m surface layer.

Mean volumetric production rates in northern Zones 1–4 ranged from lows of 10 mg $C \cdot m^{-3}$ day^{-1} to highs of 30–40. In Zones 5 and 6 in the southern basin, volumetric carbon assimilation rates were considerably higher, ranging from spring lows of 20–30 mg $C \cdot m^{-3} \cdot day^{-1}$, to midsummer and autumnal highs of 60–80.

PREDICTION OF BABINE PRIMARY PRODUCTION USING LINEAR AND MULTIPLE REGRESSION EQUATIONS

Many have attempted to calculate primary production using estimated environmental parameters (Ryther and Yentsch 1957; Schindler and Nighswander 1970; Schindler and Comita 1971). A number of variables known to be either directly or indirectly related to production were tested. The highest correlation was between areal annual production and mean Secchi depth at each station (Fig. 5). This negative relation was not surprising, for in dystrophic lakes, Secchi variation is most often related directly to the abundance of autochthonous material.

A number of variables were tested simultaneously (multiple linear regression) to find an equation that would have more predictive value than offered by simple linear regression. The most significant evaluation was obtained using the following parameters: (X_1) $\bar{x}$ Secchi, (X_2) $\bar{x}$ dry weight seston, (X_3) $\bar{x}$ chlorophyll *a*, (X_4 $\bar{x}$ phytoplankton volume, to predict (Y) $\bar{x}$ daily primary production (F values = 5.40, significant

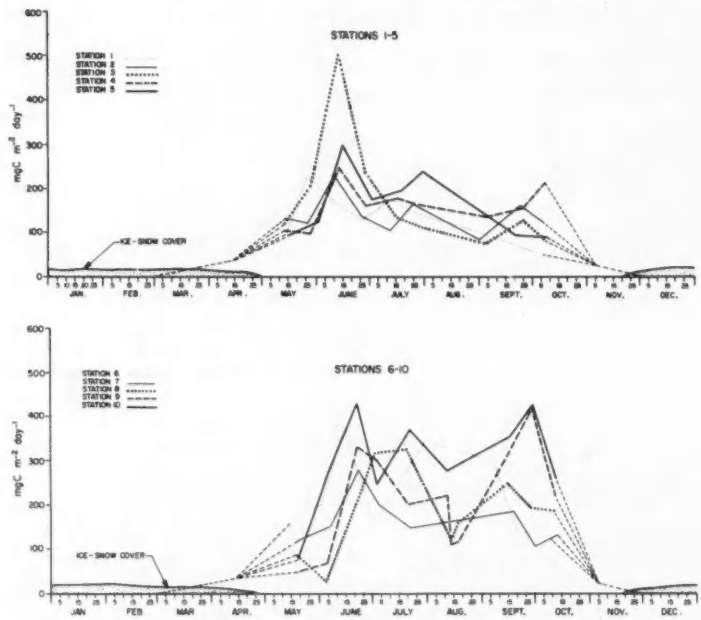

FIG. 4. Annual variation in primary production at Station 1–10, Babine Lake, 1973.

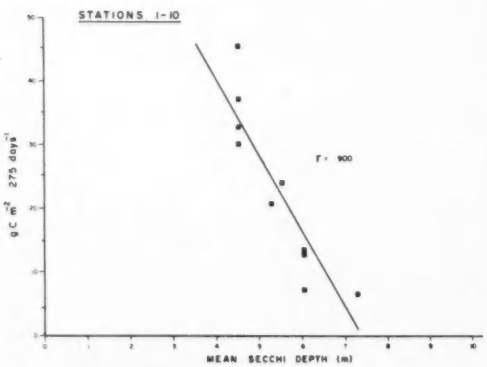

FIG. 5. Regression of annual areal production and Secchi depth, Stations 1–10, Babine Lake, 1973.

0.05 level, df = 4 and 9, coefficient of multiple determination $R^2 = .88$). The agreement between predicted values and actual observed values was excellent (Fig. 6).

Discussion

PHYTOPLANKTON PRODUCTION

In 1973 the average daily rate for ice-free season (all stations) was 122 mg $C \cdot m^{-2} \cdot day^{-1}$. In the more productive southern basin it was higher, averaging 145 for five stations (Zones 5 and 6), contrasted to only 100 for the five stations located north of Topley Landing (Zones 1–4). In 1966, in a less intensive study, Narver (1967) estimated average production to be 55 mg $C \cdot m^{-2} \cdot day^{-1}$, with highest production occurring in the south basin. Reasons for these discrepancies are discussed in detail in Stockner and Shortreed (1974).

Based on 1973 estimates, Babine can be considered more productive than many well-studied, Alaskan, sockeye-producing lakes (Harry et al. 1967). Great Central Lake, a sockeye producer on Vancouver Island, is an extremely unproductive lake when compared to Babine (Stephens et al. 1969). The average estimated daily rates vary between 1 and 5 mg $C \cdot m^{-2} \cdot day^{-1}$, which is nearly an order of magnitude less than average Babine Lake values.

On a volumetric basis (milligrams $C \cdot m^{-3}$), mean estimates for Babine were considerably higher than for Alaskan lakes (Harry et al. 1967), and an order of magnitude higher than Great Central Lake (Stephens et al. 1969).

When compared to similar estimates from other Canadian lakes, Babine Lake ranks as one of the more productive large Canadian lakes. It is not as productive as Kootenay Lake, which is of

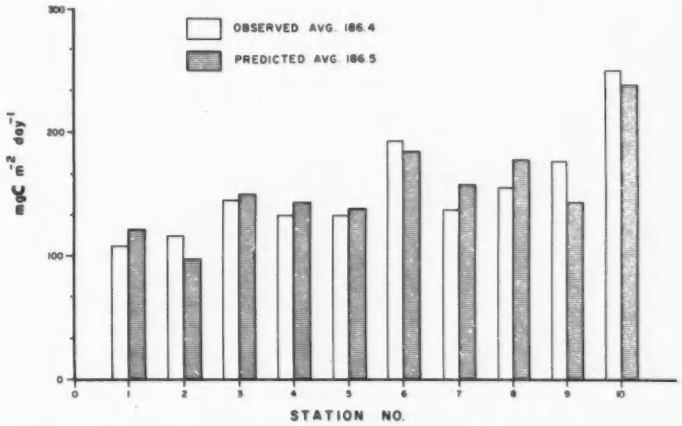

FIG. 6. Results of observed average daily production and predicted production using multiple linear regression analysis, Stations 1–10, Babine Lake, 1973.

comparable size, but Kootenay has shown greatly increased production in recent years due to the addition of considerable quantities of phosphorus (Northcote 1973). Babine is more productive than Okanagan Lake, also of similar size, but not as productive as some of the smaller eutrophic lakes in the Okanagan Basin (Stockner and Northcote 1974).

Babine Lake is more productive than most dimictic Shield lakes in the Kenora, Ontario, Experimental Lakes Area (Schindler and Holmgren 1971), but less productive than Clear Lake, situated on the Shield north of Toronto (Schindler and Nighswander 1970).

Trophic state — It is informative to compare Babine phytoplankton production to ranges established for various well-studied temperate European oligo- and eutrophic lakes (Rodhe 1969).

	Autotrophy (phytoplankton)			
	oligotrophic lakes	eutrophic lakes		
		natural	polluted	
mean rates in growing season	30–100	300–1000	1500–3000	mg of C/m² per day
annual rates	7– 25	75– 250	350– 700	g of C/m² per yr

Babine Lake, with an average daily rate of 122 mg $C \cdot m^{-2} \cdot day^{-1}$ and annual rate of 33 g $C \cdot m^{-2} \cdot yr^{-1}$, falls between ranges established for oligotrophic and naturally eutrophic lakes, albeit closer to the oligotrophic than eutrophic category. If it were not for the prevalence of humic materials staining the waters of Babine Lake, one could conclude from this, that Babine is mesotrophic, or perhaps more correctly, meso-oligotrophic.

Thienemann (1921) was one of the first to make the distinction between clear-water and brown-water lakes in his lake typology, calling the latter type dystrophic. This type of lake was typically stained with humic material leached from the watershed, and characteristically exhibited rather low production. Jarnefelt (1925) in studies on several Finnish lakes concluded that not all dystrophic lakes are unproductive; in fact, some were very productive. These productive stained lakes he termed mixotrophic, to distinguish them from Thienemann's nomenclature for the productive clear-water lake — mesotrophic and eutrophic. It appears then, on the basis for the preceding, that it would be correct to term Babine a dystrophic lake, thereby taking into consideration the stained nature of its water, and to classify it on the basis of phytoplankton production as mixotrophic.

RELATION OF PHYTOPLANKTON PRODUCTION TO PHYSICAL AND CHEMICAL FACTORS

Light — The remarkable similarity between the total radiation curves and carbon assimilation profiles when plotted an a semilog scale is suggestive of light limitation of production in the euphotic zone of Babine Lake (Fig. 7). Findenegg's (1964) description of this type of profile (Type 1) fits well with the general production profile observed at all stations in Babine. The more notable features of the Type 1 curve are: a) seasonal differences in areal production rates, but slope of production profiles remain uniform, b) light inhibition at the surface on bright days with well-developed maximums at 1–2 m, c) a

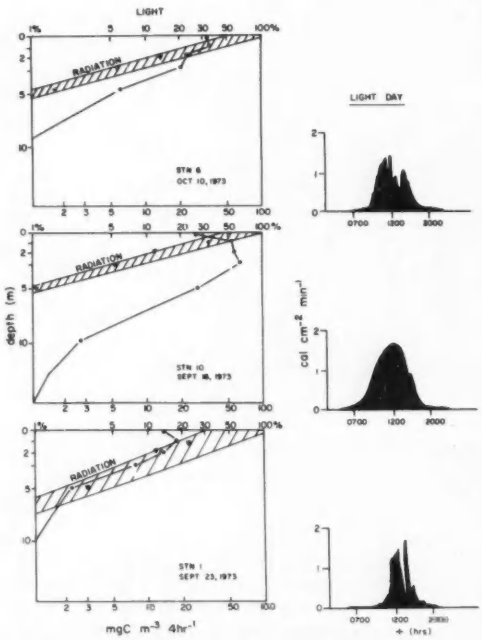

FIG. 7. Semilogarithmic plot of daily primary production and light penetration as a function of depth. Incident light on days of incubation shown on right.

rapid decline in production from 2 to 5 m, commensurate with the rapid attenuation of incident radiation in surface layers.

The nature of production profiles found in Babine is similar to the Type 1 encountered in eutrophic lakes that have an abundance of nutrients and a high standing stock of phytoplankton that self-shade to reduce light penetration. In Babine the role of stained materials must be considered as the main factor responsible for high light attenuation in surface waters, and the consequent limitation of the compensation depth (~ euphotic zone) to 5–6 m. At most stations maximum production occurred in the layer where incident radiation was reduced to about 50% of surface values (Fig. 7).

Mean Secchi disc transparency for 1973 was 5.37 m ($n = 97$), and ranged from 2.5 to 8.0 m. Data gathered in 1947 were remarkably similar, ranging from 2.2 to 6.6 with a lake average of 5.42 m ($n = 20$). This similarity indicates that transparency has changed little in 26 yr.

Nutrients — Phosphorus, nitrogen, and silicate measurements in 1973 were too infrequent to allow meaningful relations to be made between nutrients and seasonal production. There were obvious inverse relations between NO_3, SiO_2, and chlorophyll *a* concentrations and production, while PO_4 was consistently below analytical detection limits (Table 3). In 1963 and again in 1969, Stephens et al. (1969) conducted nutrient and chlorophyll *a* analyses. Measurable quantities of NO_3 were always present, even during periods of chlorophyll maxima. Lowest mean values for NO_3 and PO_4 occurred in Zone 1 and 2 and in Zone 4 opposite Old Fort Babine in the north basin of the main lake. It is reasonable to assume that phosphate, and perhaps in certain situations nitrate, limit phytoplankton growth during calm conditions when little or no mixing occurs in the epilimnion (euphotic zone). These physical conditions occur more frequently in the sheltered north basin and Hagan and Morrison arms, and it is here that nutrient limitation undoubtedly influences production the most.

Since diatoms are the dominant phytoplankton group (by volume) in Babine, silicate concentrations could conceivably limit their growth. According to Lund (1950, 1954), *Asterionella formosa* and *Melosira italica* cannot grow sufficiently to maintain populations if external supplies fall below 0.5 mg·liter^{-1}. In Babine, at most stations, values ranged from 5.6 to 1.4 mg·liter^{-1}, and never fell below 1.4 (Table 3). Though there was a measurable decline in silicate commensurate with the autumnal bloom in the southern region (values fell from 4.0 to 2.5 mg·liter^{-1}), values did not approach what could be considered critical limits for controlling growth. Thus, it appears unlikely that SiO_2 is a limiting factor to diatom phytoplankton growth in Babine.

For diatoms, carbon is the element needed in largest quantity after silica (Lund 1954). In Babine pH fluctuated from 6.8 to 7.6, and alkalinity from 30.8 to 37.5 (expressed as milligrams $CaCO_3$·liter^{-1}). It is improbable that a potential carbon supply of this magnitude is ever limiting to algal growth. Of the other major and minor ionic constituents in Babine water, none appear to be at concentrations that could be considered limiting to algal growth (Table 5).

MIXING

Mixed layer depth in 1973 showed considerable regional variation. In the North, Hagan, and Morrison arms, due to greater wind protection, a stable stratification and greatly reduced mixed layer (usually < 6 m) was typical, while from Topley Landing southward, greater exposure to strong summer winds created a less stable stratification and a greatly increased mixed layer depth (usually 15–20 m).

TABLE 5. Analysis of selected ions at four stations in Babine Lake, August 3, 1972.

		mg·liter^{-1}							
Station	Depth	*Ca*	*Na*	*Zn*	*Cu*	*Fe*	*Cd*	*K*	*Mn*
S. Pinkut	0	5.3	0.83	.04	< .01	.07	< .03	.35	.01
(Zone 6)	5	2.8	0.45	.08	< .01	.07	< .03	–	.01
	20	8.5	1.70	.02	< .01	.03	< .03	.66	.08
Port Arthur	0	9.5	1.75	.02	< .01	.03	< .03	.60	.01
(Zone 5)	5	6.3	0.91	.01	< .01	.03	< .03	.30	.01
	20	9.5	1.80	.04	< .01	.03	< .03	.55	.03
Old Fort	0	9.1	1.40	.05	< .01	.03	< .03	.55	< .01
(Zone 4)	5	9.7	1.60	.02	< .01	.04	< .03	.60	< .01
	20	9.1	1.50	.02	< .01	.03	< .03	.55	< .01
Black Point	0	9.0	1.40	.02	< .01	.03	< .03	.50	< .01
(Zone 5)	5	8.8	1.45	.02	< .01	.03	< .03	.55	< .01
	20	9.1	1.70	.02	< .01	.03	< .03	.55	< .01
	Avg	8.1	1.37	.03	< .01	.04	< .03	.52	.02

In Babine the spatial differences in mixed layer depth strongly influence phytoplankton production. There are both positive and negative effects to phytoplankton of increasing and/or decreasing the depth of the mixed layer:

Condition	Unstratified or strong mixing (deep epilimnion)	Stable stratification or little mixing (shallow epilimnion)
Light	Little surface photoinhibition Average light available at any depth (I avg) $\frac{I_o}{D}\int_o^D \exp(-kz)\,dz$	Surface photoinhibition Light depth dependent and = $I_o \exp(-kz)$ Light decreases with increasing depth
	where: I_o = light intensity at $z = 0$; D = mixed layer depth k = extinction coefficient z = depth with respect to surface	
Nutrients	No shortage	Nutrient limitation
Temp	Generally lower with uniform depth distribution	Generally higher, with greatest temperatures near surface, decreasing with depth

The lack of autumnal blooms in Zones 1–4 is likely attributable to the more stable and shallower mixed layer (5–7 m). This stable condition prevents the entrainment of sufficient nutrients from the hypolimnion to sustain an autumnal phytoplankton maximum. By contrast, in the south basin where depth and volume of epilimnion are nearly double north basin values, upwelling commonly occurs at numerous locations throughout the summer after strong wind episodes, and nutrients from entrained hypolimnetic waters, as well as those recycled from the deeper epilimnion, are of sufficient concentration to sustain an autumnal bloom of considerable magnitude, assuming sufficient light during this period. The principal algal species responsible for the fall bloom is *Tabellaria fenestrata*, a large chain-forming diatom with an average cell volume of 3000 μ^3, requiring turbulence to maintain its position within the euphotic zone. The same species exhibits lesser numbers in the lake north of Topley Landing, with the exception of Hagan Arm.

Morphometric-edaphic considerations — Other factors that may also account for regional differences in production are lake morphometric, watershed edaphic, and regional climatic influences. Babine lies in a NW–SW axis, with major outflow to the northwest. Surrounding mountain ranges are a notable feature of the north basin, creating an increase in both total precipitation and percent cloud cover. To the south of Topley Landing a more gently rolling landscape is common with thousands of acres of forest land and upland plateau. Here less cloud and precipitation occurs. These physiographic differences create small, but perhaps significant, climatological differences. The prevalence of more wind of greater velocity and its effect on lake turbulence in the south basin has already been mentioned.

Another important feature is differences in surface run-off. The main lake basin from Topley southward receives the major surface water inflow to Babine, comprising some 60–70% of total inflow. The north basin, excluding Morrison Arm, by contrast receives only 5–10% of annual inflow. Thus, differences in surface nutrient loads from these inflows contribute substantially to the total lake load which in concert with strong mixing, undoubtedly boosts production in the southern main lake region.

TABLE 6. Total phosphorus budget, 1972–73 Babine Lake. (Areal loading rate 0.05 g $TP \cdot m^{-2} \cdot yr^{-1}$.)

	$m^3 \times 10^9 yr^{-1}$	Total phosphorus ($mg \cdot m^{-3}$)	Load TP ($kg \cdot yr^{-1}$)	%TP from all sources
Inflow[a]				
Fulton R.	.6929	10.0[d]	6,929	29
Pinkut R.	.2604	10.0[d]	2,604	11
Morrison R.	.2027	7.5[d]	1,489	6
Remaining tributaries[b]	.9640	7.5[d]	7,230	30
Precipitation[c]	.2920	4.8[e]	1,392	6
Salmon carcasses[e]	–	–	4,490	19
Total	2.3000		24,134	
Outflow[a]				
Babine R.	2.1200	3.5[f]	7,428	31
Evaporation[c]	.1800			
Total	2.3000		7,428	

[a]May 1972–May 1973 (Water Survey of Canada 1974).
[b]Obtained by difference.
[c]Smith (1973).
[d]Average from low flow determinations — values increased to account for increases during spring freshet.
[e]From Donaldson (1967).
[f]Avg. from Stephens et al. (1969).

THE PHOSPHORUS BUDGET

No attempts have been made in the past to relate annual phosphorus input to trophic state of Babine Lake. A recent hydrometeorologic inventory (Water Survey of Canada 1974) provided sufficient data to compute a water balance for 1972–73, which was essential in preparation of the phosphorus budget (Table 6). It must be noted that the budget is based on relatively few stream nutrient observations and uses mean values which increase the confidence limits of estimates.

The majority of the total phosphorus input to Babine comes from the major tributaries, notably the Fulton River. Altogether, up to 81% of the total 2.41×10^4 kg was contributed by surface stream flows and precipitation; salmon carcasses contributed 19% to the total load (Table 6). It is important to note that 70–80% of this surface inflow nutrient load is received in the middle and south basins of the lake.

About 7500 kg of TP or 30% of the total load leaves the lake yearly via the Babine River, which means that about 70% is retained, the majority of this lost to the sediments.

Loading data to Babine when expressed on an areal basis and plotted on a log-log scale as a function of mean depth over theoretical residence time provides a basis for comparison of trophic state among lakes (R. Vollenweider unpublished data) (Fig. 8). Babine lies in the oligotrophic range.

ADDITIONAL EVIDENCE OF REGIONAL DIFFERENCES IN PRODUCTION

It was clear at the early stages of data analysis that the south basin of the main lake was more productive than the north basin. Results of statis-

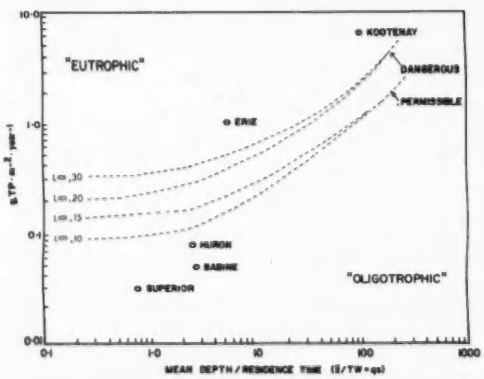

FIG. 8. Annual total phosphorus loading to Babine as a function of mean depth divided by residence time (Vollenweider 1973). Other Canadian lakes and Laurentian Great Lakes shown for comparison.

tical tests for means from Stations 1–5 in the north and 6–10 in the south have shown these regional differences to be significant (Table 7). Explanations to account for these differences based on the interplay between mixed layer depth, mean depth, nutrients, surface inflow, and the autumnal diatom maximum have already been advanced. However, if such large differences in spatial patterns of production have occurred historically, then other components of the biocoenosis should also reflect these differences. Moreover, the spatial variation of the absolute abundance of diatoms in the sediments should reflect the 1973 diatom plankton time series record, which showed higher diatom population densities at southern stations.

Zooplankton and Fish

The zooplankton and underyearling sockeye populations of Babine have been well studied (Johnson 1956, 1958; Johnson and Groot 1963; Narver 1969; McDonald 1969). No attempt will be made to review this information in any detail, rather to select information that shows regional differences in density or growth.

By the June–July period there are greater concentrations of zooplankton (milligrams·m^{-3}) at stations south of Topley Landing than in the North Arm (Johnson 1956). In 1957–58 the mid-June to mid-October zooplankton mean dry weight in the 0–5-m layer was over 100 mg·m^{-3} south of Topley Landing, but averaged less than 50 in the North Arm (Johnson 1958).

McDonald (1969) has shown that mainlake fry from Fulton River disperse rather quickly to the south basin (Zone 6) and over the summer gradually move northward, with the population center just off Fulton River in October. The northern basin of the main lake (Zone 4) had the lowest fry densities. Spring migration route of Fulton fry seems to place them in an advantageous position in the most productive region of the lake. In the past it was assumed that the carrying capacity for juvenile sockeye per unit of lake nursery area was similar throughout the lake "uniform distribution hypothesis" (Johnson 1958), but based on our findings, and on zooplankton density estimates, production per unit of lake area is unequal, being far greater in the region south of Topley Landing.

Paleolimnological evidence — If diatom production is greater in the south because of the sustainment of the autumnal bloom of *Tabellaria fenestrata* (as we have shown to be true in 1973), and if this has occurred historically for decades, then the diatom microfossil record should substantiate our 1973 findings. Figure 9 illustrates the difference in total diatom production between Station 1 (core B1) in the North Arm and Station 8 (core B8) in the south basin just off Donald's Landing. The integral value for the top 10 cm of sediment shows Station 8 to have produced over three times as many diatoms, spanning a time period of about 30 yr (Stockner 1974). Of even greater significance is the paucity of *Tabellaria* frustules at Station 1 (North Arm), 7 million cm^{-3}, as opposed to 169 million cm^{-3} of wet sediment at Station 8 (Stockner 1974). With the exception of Morrison and Hagan arms where a sizeable number of *Tabellaria* frustules appear in the sediments, no significant increase in *Tabellaria* is seen in the main lake until sediment core B6, taken just off Topley Landing. These findings substantiate our 1973 plankton counts and show rather conclusively that a regional disparity of production has occurred for decades and probably longer.

TABLE 7. Results of *t*-test statistic testing significance of north south regional production disparities (mean of Stations 1–5 vs. mean of Stations 6–10).

Parameter		"*t*" value	Significance level
1. Primary production ($g\ C{\cdot}m^{-2}{\cdot}yr^{-1}$)	8	3.11	$P = .02$
2. Phytoplankton vol ($\bar{x}\ mm^{-3}{\cdot}m^{-3}$)	8	5.09	$P = .01$
3. Chlorophyll *a* ($\bar{x}\ mg{\cdot}m^{-3}$)	8	10.08	$P = .01$
4. Seston ($\bar{x}\ g{\cdot}m^{-3}$)	8	1.71	$P = .20$

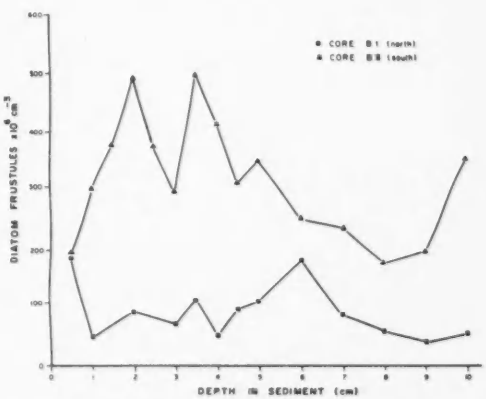

FIG. 9. Absolute abundance of diatom frustules in top 10 cm of sediment from north (B1) and south (B8) basins of Babine Lake.

Acknowledgments

We thank the Pacific Biological Station, Nanaimo, and Fisheries Operations, North, for use of their facilities. Many persons have given generously of their time to assist in various aspects of this work. Mr H. D. Smith has coordinated field operations and provided valuable information on past work on Babine, as well as a critical review of this manuscript. Mr I. Miki, F. Jordan, D. Cliff, P. Rankin, and J. Weir have assisted in field operations and have given time in aspects of laboratory analysis. Dr N. Antia and Mr J. Cheng assisted in chlorophyll *a* determinations and in scintillation counting at the Fisheries and Marine Service, Vancouver Laboratory. Dr C. Pharo has described the geological setting, Dr D. Farmer has given invaluable assistance in interpretation of physical data, and Dr M. Waldichuk has assisted in critical review of this manuscript.

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Establishment of an Asymptomatic Carrier State Infection of Enteric Redmouth Disease in Rainbow Trout (*Salmo gairdneri*)[1]

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BUSCH, R. A., AND A. J. LINGG. 1975. Establishment of an asymptomatic carrier state infection of enteric redmouth disease in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 2429–2432.

The dynamics of establishment, pathogenesis, and continued maintenance of Enteric Redmouth (ERM) disease in rainbow trout (*Salmo gairdneri*) were examined. Groups were infected by parenteral and oral routes and subsampled at weekly intervals. Both modes of infection were standardized to obtain ID_{100} levels of infection with an average of 30 bacteria constituting an LD_{50} dosage when administered parenterally. The clinical course of the disease was monitored up to 102 days postinfection. A clinically asymptomatic carrier state infection was established in the lower intestine of 25% of survivors 45 days postinfection at 14.5 C. Thereafter, regular intestinal shedding of the pathogen, causing recurrent infection and mortality within the population, occurred on a cyclic basis of 30–40-day intervals. Clinical recovery, establishment of the asymptomatic condition, and periodicity of intestinal shedding and the clinical course of the disease to convalescence were described.

BUSCH, R. A., AND A. J. LINGG. 1975. Establishment of an asymptomatic carrier state infection of enteric redmouth disease in rainbow trout (*Salmo gairdneri*). J. Fish Res. Board Can. 32: 2429–2436.

Nous avons étudié la dynamique d'infection, de pathogénie et de persistance de la maladie de l'« enteric redmouth » (ERM) chez la truite arc-en-ciel (*Salmo gairdneri*). Des groupes ont été infectés par voies parentérale et orale, et des sous-échantillons ont été prélevés de semaine en semaine. Nous avons standardisé les deux modes d'infection de façon à obtenir des niveaux de DI_{100} avec une moyenne de 30 bactéries constituant une DL_{50} lorsque administrée par voie parentérale. Nous avons suivi le cours clinique de la maladie jusqu'à 102 jours après infection. Nous avons produit une infection à l'état de porteur, sans symptôme clinique, dans l'intestin inférieur de 25% des survivants, 45 jours après infection, à 14.5 C. Par la suite, une perte intestinale régulière de l'organisme pathogène, causant une infection et des mortalités périodiques au sein de la population, se produisit de façon cyclique à intervalles de 30–40 jours. Nous décrivons le rétablissement clinique, l'établissement de la condition asymptomatique, et la périodicité des pertes intestinales et le cours clinique de la maladie jusqu'à la convalescence.

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THE asymptomatic disease carrier state in fish is important to the maintenance of fastidious pathogens within a population by acting as a constant reservoir for recurrent infection. The carrier state is also important in dissemination of disease by the transfaunation of the infected host. Consequently, the condition has been recognized as increasingly important to modern fish culture practices, management, and programs of disease surveillance, certification, and control.

Asymptomatic infection is commonly assumed to occur with both viral and bacterial fish diseases and is often used to explain pathogen dissemination or endemic infection as described for ERM[3] disease (Rucker 1966; McDaniel 1971; Wobeser 1973), bacterial hemorrhagic septicemia (Snieszko and Bullock 1968), furunculosis (Snieszko 1969), mycobacteriosis (Parisot and Wood 1966), vibriosis (Ross 1970), bacterial kidney disease (Wolf 1966), columnaris (Snieszko and Ross 1969),

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and ulcer disease (Piper 1970). The clinical picture of the carrier condition, however, is often poorly defined and understood.

Present and proposed procedures of fish disease inspection and certification are statistically based upon the suspected carrier incidence of the specific disease agent for determination of sample size (Canadian Committee of Fish Diseases 1972; Ossiander and Wedemeyer 1973), although the actual carrier incidence has yet to be established for many important pathogens, including ERM.

This study was designed to determine dynamics of establishment of the carrier state, the mechanism of maintenance, and levels of incidence in rainbow trout infected with ERM.

Materials and Methods

Etiological agent — Enteric redmouth disease was first described by Rucker (1966) and the etiological agent by Ross et al. (1966). It has subsequently been known as redmouth disease, red vent disease, bacterial hemorrhagic septicemia, and Hagerman redmouth (HRM) disease. Fifty-three isolates of wide host range and geographical distribution were collected and compared on a biochemical, antigenic, and morphological basis. A single isolate, representative of the majority and previously described by Wobeser (1973), was used in these studies. Maximum virulence was maintained by four serial passages through the experimental animal prior to each use.

Experimental animal — Yearling rainbow trout lacking a prior history to or discernable clinical or serological evidence of ERM exposure and without any obvious disease condition or record of chemotherapy treatment for a period of 30 days prior to infection were used in all experiments. Experimental fish averaged 24 cm in fork length and 185 g in weight. All experiments were conducted in 756-liter circular self-cleaning fiberglass tanks with a partial recirculating system using 7.6 liter/minute make-up water. Recirculated water was filter clarified to 15 μm ("Fulflo" honeycomb particulate filter, Commercial Filters Corp., Lebanan, Indiana) and passed through an ultraviolet filter (Model S1-1, Aquafine Corp., Los Angeles, Calif.) at 37.8 liter/minute with 25,000 μW seconds exposure. Water temperature was held at a constant 14.5 C.

Mode of infection — One hundred and fifty-four fish were parenterally infected by intraperitoneal (IP) injection of an average of 18 bacteria harvested from an 18-h trypticase soy agar (TSA) culture and resuspended in 0.5% tryptone. Using a duplicate holding system, a second group of fifty fish was infected by means of an oral heat-stressed method. The fish were first crowded to 178 liters of water and the temperature raised to 20 C over a 1-h period. The 18-h harvested culture of the ERM bacterium was then added to give a final water concentration of 2.75×10^8 bacteria/milliliter. Following 1-h pathogen exposure at 20 C, the tank was filled to original capacity, cooled to the experimental temperature, and the water recirculated through the filter system for clearance of the pathogen.

Clinical examination — Dead fish were collected daily and mortality was recorded as a percent of the surviving population. Weekly subsamples of four or five fish were taken from the survivors of each of the two populations and killed. Clinical signs and gross pathology were noted and recorded on a positive (+), questionable (±), or negative (−) scale as described by Rucker (1966) and Wobeser (1973). Inoculating loop stabs of anterior kidney tissue and lower intestine as well as spleen and liver section swabs were used to inoculate plates of TSA. Cultures were incubated at 22 C for 48 h and isolates confirmed as ERM by rapid slide agglutination tests. Relative rates of recovery of the pathogen from various tissues were recorded as being heavy and having profuse growth (3+), medium and having 10–20 colonies per plate (2+), light and having 1–10 colonies per plate (1+), or negative (−) from each of the above indicator tissues sampled.

Results and Discussion

Mortality rate — Initial mortalities in the parenterally infected population began on day 6 postinfection, reached a maximum on day 9, and cleared temporarily on day 15 postinfection as shown in Fig. 1. Recurrent mortalities were then observed between 59 and 75 days postinfection. The experimental mode of infection resulted in a total LD_{26} for 18 bacteria administered intraperitoneally.

Heat-stressed fish exposed to the pathogen in water resulted in an LD_2 rate of total mortality after 7 days postinfection as shown in Fig. 1. Both methods of exposure produced infection of all fish at 21 days as judged by the successful recovery of pathogen from all fish sacrificed (Fig. 1). Oral exposure studies by Ross et al. (1966) indicated that mortalities first appeared at 5 days postinfection, reached a maximum rate at 10 days, and ended 19 days postinfection. These established lethal and infective dosages demonstrate the high degree of virulence of the ERM bacterium compared with many other fish pathogens and bacterial pathogens in general (Klontz 1966).

Pathological changes — Gross pathological changes of both orally and parenterally infected dead and moribund fish were commonly characterized by subcutaneous hemorrhage in and about the oral cavity, at the base of the pectoral, pelvic, and anal fins, and about the vent. Small 1–2-mm open hemorrhagic lesions were also noted about the anterior lateral aspects of the body. Exoph-

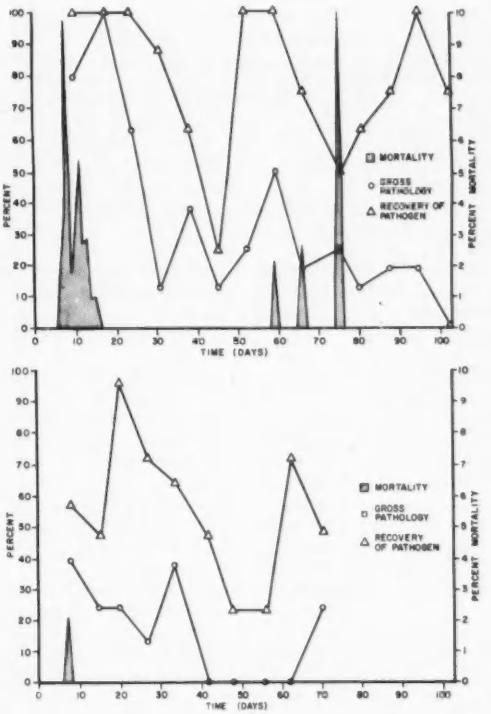

FIG. 1. Mortality, gross pathology, and recovery of ERM bacterium during periodic sampling following intraperitoneal infection (*upper*) and oral infection (*lower*) of rainbow trout (*Salmo gairdneri*).

thalmos was commonly observed, being initially unilateral and eventually bilateral, with eventual rupture of the eyeball noted. Internal gross pathology was characteristic of a massive hemorrhagic septicemia.

Relative pathogen recovery rates — During the first 30–60 days postinfection, the infectious agent was readily recovered from all indicator tissues sampled (Fig. 2). The ERM bacterium as shown in Fig. 1 and 2 readily established an asymptomatic carrier state infection in rainbow trout. The pathogen was found to localize in the lower intestine of 50–75% of survivors, 60–65 days following infection. These surviving asymptomatic fish had the demonstrable potential for transmission of the disease to other susceptible populations for more than 102 days following infection. From the data on pathogen tissue localization and relative recovery rates in Fig. 2, it was shown that the pathogen was routinely isolated from the infected populations but only from the lower intestine during the carrier state of convalescence. The relative intestinal presence of the pathogen within the infected populations was also of a cyclical nature. Therefore, only 25–50% of the asymptomatic carriers established would be clinically identified by using classical attempted isolation from the kidney and spleen at the low points of the cycle (Fig. 2). It was concluded that the organism would be difficult to isolate during inspection and certification programs using classical methods, especially if sampling only the kidney and spleen.

On the basis of pathogen recovery, gross pathological changes, and mortality rates, it appears that a regular 36–40 day cycle of intestinal shedding of the ERM bacterium occurs and preceeds the reappearance of gross pathological changes and mortality by 3–5 days. This cyclical shedding could precipitate continuous recurrent mortality in a naturally infected susceptible population throughout the year. The actual periodicity

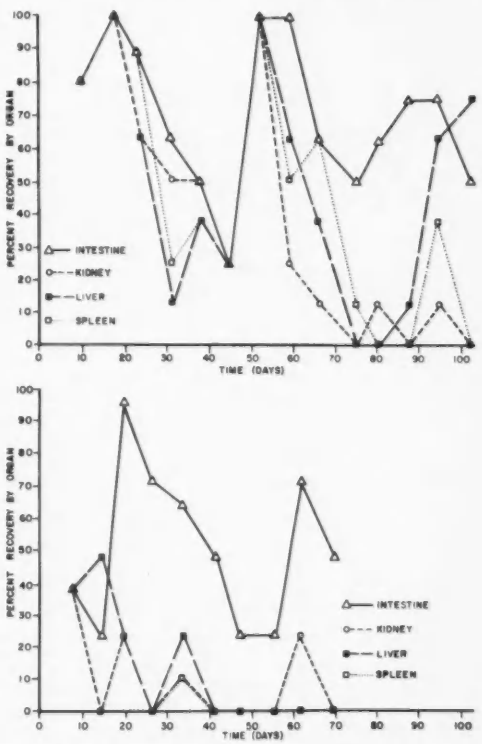

FIG. 2. Relative recovery rates of ERM bacterium from indicator organs during periodic sampling following intraperitoneal infection (*upper*) and oral infection (*lower*) of rainbow trout.

and mortality levels of the cycles could be altered by seasonal variations in water temperatures, loading factors, handling, and other stresses as well as natural resistance and immunity of the population. This hypothesis is substantiated by McDaniel (1971) who showed yearly cyclical mortality patterns of similar periodicity in a large untreated hatchery population chronically infected with ERM disease.

This study indicates that enteric redmouth disease readily establishes a convalescent asymptomatic carrier infection localized in the lower intestine of more than 25% of the fish surviving 45 days postinfection at 14.5 C. Attempts at inspection and certification of these stocks should, therefore, include sampling from the lumen of the lower intestine as the agent is not routinely present in other tissues at this time. Populations of fish with a clinical history of enteric redmouth are capable of shedding the pathogen with infected feces and could serve as a source of recurrent infection to themselves and other susceptible populations for long periods of time on a regular cyclical basis.

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Growth in a Population of the Crayfish *Pacifastacus leniusculus* from a Subalpine Lacustrine Environment

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FLINT, R. W. 1975. Growth in a population of the crayfish *Pacifastacus leniusculus* from a subalpine lacustrine environment. J. Fish. Res. Board Can. 32: 2433–2440.

Observations on growth increments at each molt in a population of *Pacifastacus leniusculus* from a subalpine lake were made from periodically recaptured marked crayfish, as well as from molts recorded in the laboratory. After the first growing season the growth increment at molt remained relatively constant. Throughout the life of this crustacean no differences were observed between sexes. Analysis of size distributions in samples taken at different times of the year revealed that between 8 and 12 year-classes were normally present in the population of Lake Tahoe. Growth of these age-classes could be followed through consecutive samples. The number of molts completed by each year-class was found by relating data on growth increment per molt to growth shown in consecutive quantitative samples. Mean carapace length at recruitment for juvenile crayfish was 3.75 mm with 11 molts occurring during the first growing season, and juveniles reached 13.0 mm by the end of their first year. Molt frequency decreased in successive year-classes, and crayfish surviving past their fourth growth season molted only once a year. Length–weight relationships were similar for both sexes of the population even though their external anatomy differed. Growth comparison between this population and that of a coastal stream population showed that the stream population had a slightly faster overall growth rate due to higher water temperatures and a longer growing season.

FLINT, R. W. 1975. Growth in a population of the crayfish *Pacifastacus leniusculus* from a subalpine lacustrine environment. J. Fish. Res. Board Can. 32: 2433–2440.

Nous avons mesuré l'accroissement de taille à chaque mue dans une population de *Pacifastacus leniusculus* d'un lac subalpin, à partir d'individus marqués recapturés périodiquement et au cours de mues observées au laboratoire. Après la première saison de croissance, l'accroissement de taille au moment de la mue est relativement constant. Nous n'avons pas décelé de différences entre les sexes durant toute la vie de ce crustacé. L'analyse de la répartition des tailles dans les échantillons capturés à différentes époques de l'année révèle que 8 à 12 classes d'âge sont normalement présentes dans le lac Tahoe. On peut suivre la croissance de ces classes d'âge dans des échantillons consécutifs. Nous avons déterminé le nombre de mues complétées par chaque classe d'âge en établissant la relation entre les accroissements de taille par mue et la croissance observée dans chacun des échantillons qualitatifs consécutifs. La longueur moyenne de la carapace au moment du recrutement est de 3.75 mm pour les jeunes écrevisses, 11 mues se produisant durant leur première saison de croissance. Ces derniers atteignent une longueur de 13.0 mm à la fin de la première année. La mue devient moins fréquente chez les classes d'âge successives. Les écrevisses qui survivent jusqu'à la quatrième saison de croissance ne muent qu'une fois par année. La relation longueur–poids est la même chez les deux sexes, même si leur anatomie externe diffère. Une comparaison de la croissance de cette population avec celle d'une population d'un cours d'eau côtier démontre que cette dernière a un rythme de croissance général légèrement plus rapide, par suite de températures de l'eau plus élevées et d'une saison de croissance plus longue.

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THE freshwater crayfish *Pacifastacus leniusculus*, besides being highly prized by Europeans for its food value, has been the focus of recently developed fisheries in the delta waters of the Sacramento River (Nicola 1971). The potential of this organism for either intensive or extensive aquaculture is presently being investigated by a number of laboratories (e.g. Sweden, British Columbia, and California).

Pacifastacus leniusculus was introduced into California from Oregon (Riegel 1959) and is

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now widely distributed throughout the northern and central parts of the state in a diversity of habitats. Since introduction, it has thrived in streams, impoundments, and lakes and has become the dominant benthic organism of many subalpine lakes of the Sierra Nevada Mountains (e.g. Donner Lake and Lake Tahoe). Crayfish play a major role in the dynamics of many aquatic communities that they inhabit (Abrahamsson 1966; Momot 1967; Dean 1969; Abrahamsson and Goldman 1970). This stimulated an extensive investigation into the population parameters and the role of this abundant invertebrate in the Lake Tahoe ecosystem.

To improve upon cultural techniques one must define the growth of the crayfish in its natural environment. Length and weight increases are related to the age and size composition of the natural population at different times of the year. With these data the growth history of a crayfish cohort is described.

Materials and Methods

The growth dynamics of *P. leniusculus* were investigated in Lake Tahoe, California from March 1974 to February 1975. Tahoe, an extremely oligotrophic subalpine lake, has a small littoral zone and averages 313 m in depth. Water temperatures ranged from a summer high of 18 to approximately 4.8 C in the winter.

Two transects were selected on the west shore of the lake, each positioned with a sextant using shore landmarks. Monthly trapping of crayfish occurred from March through June, biweekly between July and October, and monthly again from November to February. Adults were caught in baited traps made of nylon mesh intermittently spaced (5–15 m apart) on a nylon line. These traps were usually retrieved from the lake bottom between 0600 and 1100 h. Juvenile crayfish (less than 25 mm carapace length) were collected by SCUBA divers randomly searching the bottom with dipnets. Shallow portions of each transect were examined by diving during the warmer months of the year to retrieve juveniles.

For each crayfish I recorded: 1) Carapace length, from the back of the eye socket to the base of the carapace, to the nearest 0.05 mm with a Vernier caliper. All lengths used in this paper refer to carapace length (C.L.) in millimeters. 2) The sex of each animal. Sexual characteristics in crayfish less than 1 yr old are not easily identified. 3) Weight to the nearest gram. 4) Depth at which each crayfish was caught.

A marking program was conducted from March to September. The cauterization technique of Abrahamsson (1965) was used to mark approximately 5500 crayfish. This destroys the pigment of the exoskeleton and pigment-producing cells. Clipping techniques or tags are either lost after molt or interfere with the animal's activity. Cauterization, however, causes no unnecessary injury and leaves well-delimited marks, even after two molts. After marking, all crayfish were returned to the location of their capture. Subsequent recapture of marked crayfish produced estimates of individual growth after molt. Additional crayfish nearing ecdysis (red abdomen or distinct separation between abdomen and carapace) were brought into the laboratory to obtain molt data. To eliminate influence of artificial conditions on growth increments, only crayfish that molted within 5 days of capture were included in the results (Hopkins 1967).

Growth was determined both from the relationship of premolt–postmolt carapace length measurements and the use of length-frequency histograms (Fig. 1A) (Van Deventer 1937). Statistical analysis of the size frequency distributions was carried out on semilog paper by the method of Tanaka (1962). Using the formula,

$$\text{Log } f(X) = -(0.217\,X^2/\sigma^2) + \text{Log } (N/2\pi\sigma)$$

where N equals the total number of animals captured and X represents the size-class (C.L.), and varying the standard deviation (σ) of mean increase at each molt by 0.12 increments (Tanaka 1962), a series of parabolas was obtained from normal frequency distributions (Fig. 1B). When these were applied to the size frequencies of the crayfish plotted on semilog paper (Fig. 1C) it was possible to analyze the polymodal distributions of the population into their age components (Fig. 1D). Significance tests were performed on age-classes of consecutive samples, both before and after the summer molting season, to check the validity of difference at the 5% level between the mean lengths.

Results

Male crayfish differ from females in having larger claws and narrower abdomens. Upon

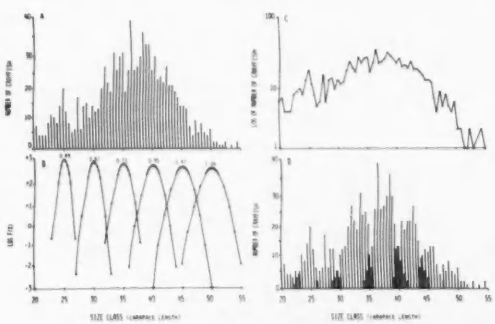

FIG. 1. Statistical analysis of length-frequency distributions of the *Pacifastacus leniusculus* population in Lake Tahoe according to the method of Tanaka (1962). A, Normal size frequency distribution of crayfish caught August 19, 1974; B, plot of lof $f(X)$ calculations (see text) varying the standard deviation by 0.12 increments. Each standard deviation indicated above its curve; C, Log plot of the number of crayfish in each size-class from A; D, Size-frequency distribution of crayfish from A after analysis as described in the text.

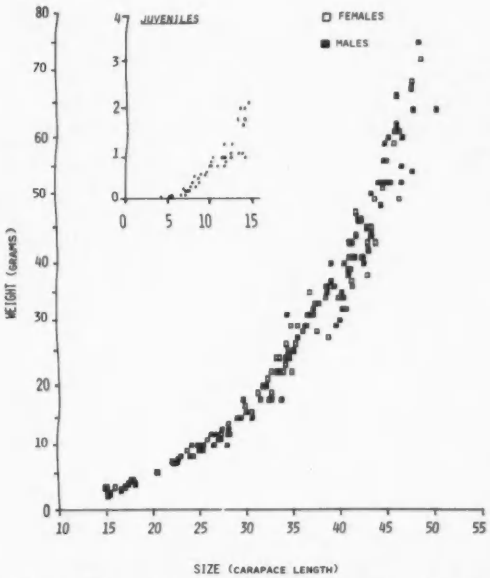

FIG. 2. The length–weight regression analysis for juvenile and adult crayfish from Lake Tahoe. Each point represents the measure of one animal. Juvenile crayfish plot represented by insert to figure.

maturing, the males' chelae show allometric growth while the females' chelipeds remain isometric throughout life (Mason 1975). In addition, females undergo a maturation molt involving an abrupt increase in the width of their abdomen (Mason 1974).

The differences in secondary sexual characteristics could result in a considerable difference in the length–weight relationship between sexes. A random sample of crayfish from Lake Tahoe did not show a significant difference (5% level) between male and female length–weight regression coefficients. A dissimilarity was seen, however, between crayfish less than 15 mm carapace length and those larger than this size (Fig. 2). The resultant regression equations for the two size-groups were as follows:

$$\text{(Crayfish less than 15 mm): Log (W)} = -3.413 + 3.202\ \text{(C.L.)} \qquad r = 0.977$$

$$\text{(Crayfish greater than 15 mm): Log (W)} = -2.958 + 2.815\ \text{Log (C.L.)} \qquad r = 0.993$$

The positive allometric growth of male *P. leniuscus* negated the increased abdomen width of females. This resulted in similar length–weight regression coefficients for the two sexes.

Annual growth in P. leniusculus is the product of a growth increment at each molt and the molting frequency of the individual; hence it is stepwise rather than continuous. Understanding the dynamics of growth requires an evaluation of the molt increments of different sized individuals and the frequency of molting over an annual cycle. For several weeks preceding molt in crayfishes, calcium is withdrawn from the old exoskeleton and stored in the form of gastroliths in the anterior wall of the stomach. The overall size of the gastrolith increases as ecdysis nears (Stephens 1955).

Consequently the width of gastroliths from crayfish was measured and plotted against time of the year (Fig. 3A). The mean gastrolith width for crayfish taken during the winter was 0.2 mm. From July through the end of August this width never dropped below 2.0 mm. Of the 150 marked crayfish recaptured, 49 showed an increase in carapace length, indicative of at least one molt. The time of marking was plotted against time of recapture for the 49 molted crayfish as well as for those that had not molted prior to recapture (Fig. 3B). There was a definite delineation between the period of high molt frequency (June–September) and the period of the year when this species failed to molt. Analysis of recapture data implied that crayfish greater than approximately 33.0 mm did not molt more than once a year.

Molt increments obtained from mark-recapture information combined with laboratory molt data and in situ measurements of molt increases (using SCUBA) provided sufficient information to establish linear regressions of premolt–postmolt growth curves (Fig. 4). Regression coefficients were calculated for male growth, female growth, and growth curves of crayfish (both sexes) on each transect. A test of homogeneity of these coefficients indicated no significant difference between male and female length increases or between growth of crayfish from the two trapping areas (both at the 5% level of significance). A curve was fitted to the premolt–postmolt data for both sexes according to the equation,

$$Y = 2.452 + 0.993\ X$$

where Y represents the postmolt carapace length and X equals the premolt size. The correlation coefficient for this first degree linear regression was $r = 0.996$. Crayfish smaller than 12.0 mm C.L. (young-of-the-year) were omitted from this plot because their molt increment was dissimilar to crayfish larger than 12.0 mm. Furthermore, sufficient data from young-of-the-year molts was lacking.

The lack of premolt–postmolt data on the smallest juvenile size-classes, led me to use the

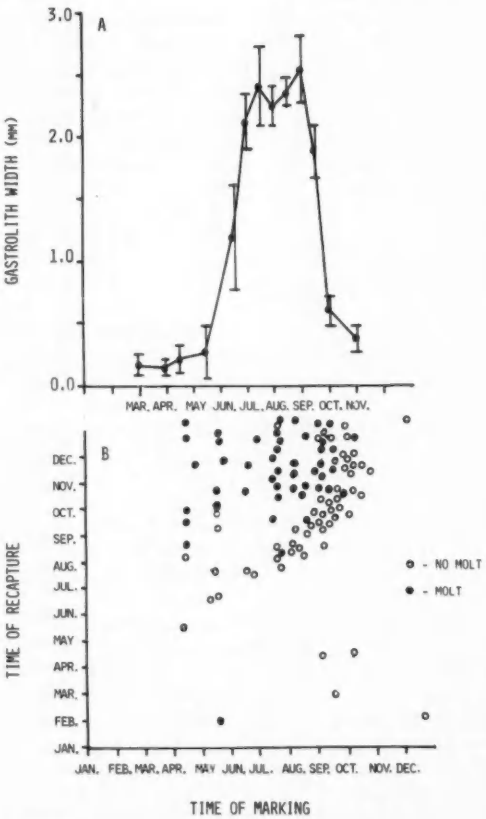

FIG. 3. A, Mean width of crayfish gastroliths measured during this investigation. The vertical bars represent a standard deviation from the mean. B, The distribution of molts, determined from size increase, of marked crayfish recaptured during this study in Lake Tahoe. Crayfish that had not molted prior to recapture are also indicated on the scatter diagram.

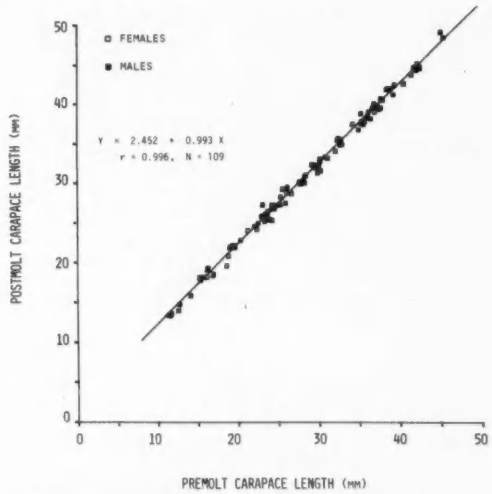

FIG. 4. Linear relationship (growth curve) between premolt and postmolt carapace length of *Pacifastacus leniusculus* in Lake Tahoe. There is no significant difference between male and female increases.

length-frequency method of Tanaka (1962). Two distinct juvenile age-classes were discovered by hand collections, using SCUBA, in the rock zones of the transects (Fig. 5). The increase in growth throughout the summer and fall months was followed quite easily. The size of young-of-the-year crayfish found in the rocks and boulders immediately after recruitment (July 22, 1974) was 3.50–3.75 mm. At the end of October the age 0 crayfish ranged in size from 6.50 mm to 10.25 mm (mean of 8.50 mm). Judging from the size ranges from the June sampling period, these age 0 crayfish would reach a mean of 13.0 mm by the end of their first year.

Age I crayfish showed a size range of 9.5–18.5 mm (mean of 14.5 mm) in July and increased to a mean of 17.5 mm by the end of October. At the end of the second year these organisms had reached a mean size of 20.5 mm carapace length. The length-frequency distributions indicate a large variation in growth among individuals. The minor modes greater than 20 mm in the distributions from August 5 through the end of October represented smaller individuals from age-class II.

Quantitative trap catches taken from the two transects in June, July, September, and October of 1974 were also analyzed by the Tanaka (1962) method. Most samples consisted of crayfish representing 12 age-classes from 0 to age XI inclusive (Table 1). The October sample displayed only 11 classes. Age classes IX, X, and XI represented less than 1% of the number of crayfish from the entire sample. In fact, these larger crayfish might simply represent spillover individuals from preceding age-classes that have maintained consistently higher growth rates. With the exception of the first three year-classes, no significant difference (5% level) occurred in mean length for age components between October and June. Growth in size was significantly different between various classes of consecutive samples ($P < .05$).

Ebert's (1973) growth rate model was used to simulate the mean individual size of the mixed age crayfish population over an annual cycle. The model required: maximum size of an individual in the population (57.0 mm); size at recruitment

TABLE 1. Mean carapace length of age components in the crayfish (*Pacifastacus leniusculus*) population from Lake Tahoe. Quantitative samples taken at 4 times during the year were analyzed by the length-frequency technique of Tanaka (1962). Figures in parentheses indicate total number of crayfish in each sample. Starting point of each year-class assumed to be July. Crayfish from age-classes 0 and I are included even though not sampled by baited net traps.

	Sampling period							
Year-class	July 1974	SD	Sept. 1974	SD	Oct. 1974	SD	June 1974[a]	SD
0	3.75	0.21	7.75	0.61	8.50	0.49	13.00	0.73
I	14.50	1.45	16.50	1.57	17.50	1.45	20.50	1.21
II	22.50	1.09	27.00	0.85	28.00	1.45	29.00	1.09
III	30.00	1.31	33.00	0.97	33.00	0.85	33.50	0.61
IV	34.50	0.49	35.50	0.85	36.50	0.73	36.00	0.49
V	36.00	0.73	38.00	0.85	38.50	0.61	38.00	0.61
VI	37.50	0.73	41.50	0.49	41.50	0.49	41.00	0.49
VII	40.50	0.61	43.50	0.73	43.50	0.61	43.50	0.49
VIII	44.50	0.49	46.00	0.61	45.50	0.61	46.00	0.49
IX	46.00	0.73	48.50	0.85	48.00	0.61	48.00	0.49
X	48.00	0.61	50.50	0.97	50.50	0.85	50.50	0.61
XI	50.00	0.61	53.50	0.85	—	—	53.00	0.49
	(330)		(887)		(1429)		(1577)	

[a]Sampling dates not placed in order because recruitment occurs in July, and June is the end of a year-class for *P. leniusculus*.

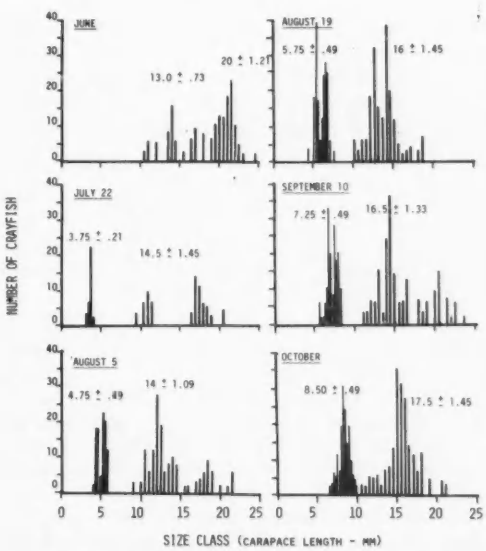

FIG. 5. Length-frequency distributions of juvenile crayfish collected by SCUBA divers in Lake Tahoe during summer 1974. Sampling intervals indicated on each graph. Mean carapace length and standard error are also indicated for each age-class in the sample.

(3.75 mm); and mean size at two times during the year. Trapping data and hand collections of juveniles supplied mean estimates of size for the entire population of 26.0 mm following recruitment in July and 30.70 mm in June, just prior to the next recruitment interval. The resultant population growth simulation from one recruitment period to the next (1 yr) is illustrated in Fig. 6. Most growth in the population occurs in the summer and early fall and ceases in the winter. Growth resumes in late spring with increasing water temperatures. Juvenile molting is primarily responsible for the further increases in size through June. Although a model, this simulation presents detailed growth of *P. leniusculus* over an annual cycle and is in agreement with earlier data (Fig. 3A and B).

The growth pattern for Lake Tahoe crayfish can be constructed (Table 2) from the linear regression formula for growth (Fig. 4). These size estimates agree with the observed age-classes in the lake (Table 1). Stage numbers 2 through 10 of this estimate are extrapolated from the length-frequency distributions of young-of-the-year (Fig. 5). Table 2 also includes the results from Mason's work (1975) on growth of the coastal stream population of *P. leniusculus*. A comparison of his estimates with the estimates for the Tahoe population indicates that Tahoe crayfish exhibit a slightly higher growth but less molt frequency through age-class II. After this age the coastal population grows at a higher rate than the Lake Tahoe population.

Crayfish in age-class 0 completed approximately 11 molts during their first growing season, with a reduction in molt frequency occurring in each successive year-class (Table 2). Age-class I cray-

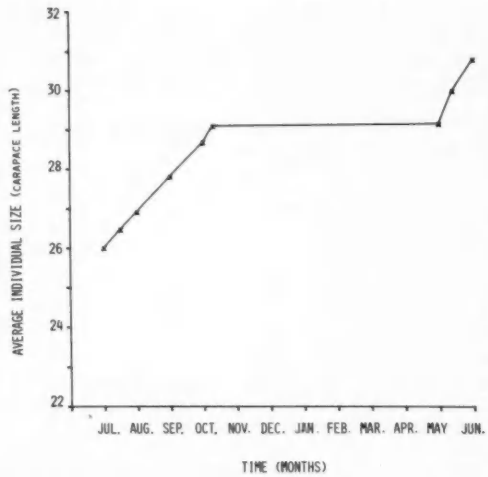

FIG. 6. Average individual size of mixed age-classes of *Pacifastacus leniusculus* over an annual cycle, beginning from recruitment. Sizes determined from computer simulation of growth (Ebert 1973).

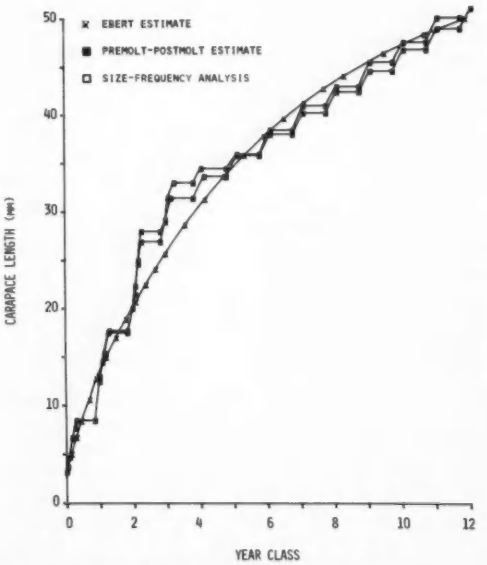

FIG. 7. Postulated growth history of a cohort of Lake Tahoe crayfish according to three different methods of calculation (see text).

fish completed four molts during their growing season, while age-class II crayfish displayed three molts and age-class III animals two molts. All crayfish surviving after their fourth growing season (year-class III) molted once a year. Using the above, I constructed the probable cohort growth history of this crayfish in Lake Tahoe (Fig. 7). The postulated growth is estimated by three different methods: 1) a computer simulation of the Von Bertalanffy growth curve formula (Ebert 1973); 2) molt increments from the premolt–postmolt linear regression; 3) and growth as estimated from length-frequency analysis. The curves for methods 2 and 3 are essentially the same throughout most of the cycle. The computer simulation (method 1) which is merely a mean estimate over the year, provides a different estimate from the other two methods. Hence, Von Bertalanffy growth does not truly represent the actual growth parameters of the crayfish because it assumes that an organism approaches its maximum size asymptotically. Crayfish, however, display stepwise linear growth throughout their life cycle, and so, as the other two methods illustrate, could grow indefinitely.

Discussion

Several investigators (Svardson 1949; Thomas 1958; Simpson 1961) have found that growth increments in decapods differ significantly between sexes. Usually females lag because of reproduction and less frequent molting. No statistically significant difference was noted, however, in growth increment between the sexes in *P. leniusculus*. This is because in females all the growth takes place during a period of the year when they are egg-free. Therefore, both males and females frequently molt during peak intervals for growth (i.e. summer).

Mason (1975) found differences in length–weight relationships between males and females in a coastal population of *P. leniusculus*. However, in Tahoe no real difference between sexes was found (Fig. 2). One reason for the difference in the coastal crayfish was that Mason plotted total length against weight, while I used carapace length for the Tahoe crayfish. A definite linear relationship exists, however, between carapace length and total length (Fig. 8). Another reason may be that Mason said that males and females were similar in weight up to 40 mm total length. Figure 8 shows that 40 mm total length approximates 16 mm carapace length. The variation in length–weight regression in Lake Tahoe crayfish is not between males and females, but rather between crayfish less than approximately 15 mm carapace length and those greater than this size (Fig. 2). Thus the length–weight relationship changes at the 15–16 mm size range and the

TABLE 2. Estimated number of age-classes, seasonal distribution and frequency of molting, and mean growth increments for the Lake Tahoe crayfish population. Growth increment estimates calculated from linear regression (Fig. 4). Last two columns show Masons' estimates (1975) for growth on the same species from a coastal stream habitat. (Su = summer, F = fall, Sp = spring.)

Year-class	Number molts	Stage number	Season of year	Carapace length (mm)	Mason age-class	Mason C.L.[a]
		2[b]	Su	3.25		3.74
		3	Su	3.75		4.20
		4	Su	4.37		4.71
		5	Su	5.25		5.27
0	11	6	Su	6.20	0	5.89
		7	F	7.50		6.56
		8	F	8.40		7.31
		9	Sp	9.52		8.13
		10	Sp	11.18		9.03
		11	Sp	12.95		10.03
		12	Su	15.36		11.13
I	4	13	Su	17.71		12.37
		14	F	20.03	I	13.75
		15	Sp	22.35		15.30
		16	Su	24.64		17.05
II	3	17	F	26.92		19.06
		18	Sp	29.18		21.37
III	2	19	Su	31.43	II	24.03
		20	Su	33.66		27.05
IV	1	21	Su	35.88		30.46
V	1	22	Su	38.08		34.18
VI	1	23	Su	40.27	IV	37.54
VII	1	24	Su	42.44	V	40.35
VIII	1	25	Su	44.59	VI	42.74
IX	1	26	Su	46.73	VII	44.78
X	1	27	Su	48.86	VIII	46.55
XI	1	28	Su	50.97		

[a]Mason's data was in total length. Converted to carapace length according to, Total Length = 2.201 + 2.27 (Carapace Length), Fig. 8.

[b]Length of first stage unknown because young still attached to female pleopods. Recruitment takes place after molt to third stage.

difference between coastal and Tahoe adult populations may reflect differences in environmental parameters such as temperature and food.

Environmental parameters are probably also responsible for differences in growth (length increases) between the coastal stream and Lake Tahoe populations. Svardson (1949) notes that higher temperature increases the molt increment and frequency of *Astacus astacus*. The higher annual water temperatures of the coastal stream habitat (Mason personal communication) may cause a larger increment at each molt. This explanation however, doesn't account for the slower growth of juvenile crayfish in the coastal environment. Though molting is more frequent in the coastal population because of higher temperatures, the juveniles are found in flowing waters (Mason 1975) where the environmental stress may be greater and cause smaller growth increments at each molt. Crayfish of the genus *Paranephrops* found in deep pools of streams were generally larger than those found in shallow riffle portions of the same stream (Hopkins 1967). The environmental stress from flowing waters in the coastal stream habitat causes the juveniles to expend more energy in maintaining their position. Therefore, they show smaller growth at each molt than Tahoe crayfish inhabiting calm nearshore waters of the lake.

The Ebert growth simulation for the Tahoe crayfish (Fig. 7) cannot be completely disregarded because it is relatively close to observed data for later age-classes. Where the researcher lacks the appropriate data to determine growth, the Ebert model could prove suitable as a population growth model (Fig. 6) and provide a fairly realistic representation of a species' growth dynamics. All three models of the growth of a crayfish cohort are valuable for both management and culture purposes. More importantly the three different

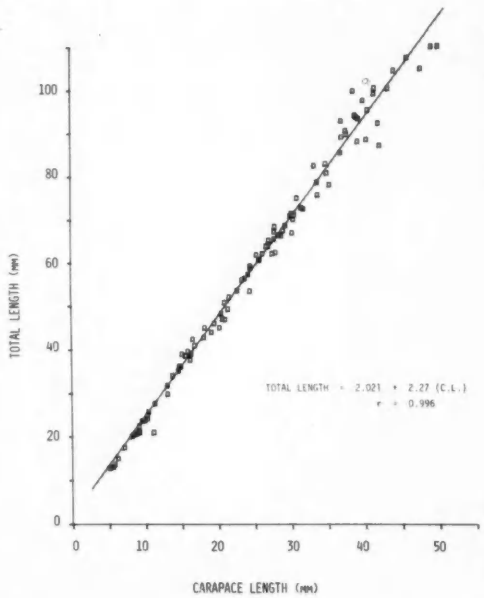

FIG. 8. Linear regression of total length vs. carapace length for Lake Tahoe crayfish.

techniques prescribe very similar growth patterns. Hence, growth obtained from the statistical analysis of population frequency distributions, an often maligned technique in population biology, can truly represent the growth pattern of the Lake Tahoe population of *P. liniusculus*.

The crayfish, *P. leniusculus*, is a very prolific invertebrate. It even grows and reproduces well in Swedish lakes (Abrahamsson 1971). The results presented here imply that this species adapts to different habitats without any significant change in its growth patterns. There are many biotopes suitable for this species and many environments not yet reached by this crayfish. With the capabilities of *P. leniusculus* to adjust to many different biotopes, this species is a prime candidate for introduction as well as for artificial manipulation through aquaculture programs.

Acknowledgments

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Possible Role of the Posterior Lateral Line in Obstacle Entrainment by Brook Trout (*Salvelinus fontinalis*)

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SUTTERLIN, A. M., AND S. WADDY. 1975. Possible role of the posterior lateral line in obstacle entrainment by brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 32: 2441–2446.

Observations of position holding by brook trout (*Salvelinus fontinalis*) in a stream channel indicate that they choose specific locations probably related to flow patterns around bottom obstructions. Bilateral denervation of the posterior lateral line system of trout has no effect on their ability to entrain on objects placed in flowing water, providing sufficient visual cues are available. Unilateral ablation resulted in a lateral bias in swimming position relative to the flow obstruction even when visual cues were present. Bilateral denervation of the posterior line system reduced the degree to which trout could entrain on objects when visual cues were omitted. It is suggested that in stream-dwelling fish, such as the brook trout, the lateral line may serve as a detector of flow or pressure discontinuities, enabling the fish to maintain position with minimum expenditure of energy.

SUTTERLIN, A. M., AND S. WADDY. 1975. Possible role of the posterior lateral line in obstacle entrainment by brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 32: 2441–2446.

Des observations effectuées sur la façon dont des ombles de fontaine (*Salvelinus fontinalis*) font pour demeurer au même endroit dans le courant indiquent que celles-ci choisissent des endroits qui sont probablement reliés au type d'écoulement de l'eau contournant les obstacles situés sur le fond. L'énervation bilatérale de la partie postérieure du système de la ligne latérale n'a eu aucun effet sur leurs capacités de s'aligner sur des objets placés dans le courant, à condition que des points de repère visuels suffisants soient disponibles. L'ablation unilatérale a provoqué un déplacement latéral de la position de nage relativement à l'obstacle, même si des points de repère visuels étaient disponibles. L'énervation bilatérale postérieure a provoqué une réduction de la capacité des truites de s'aligner sur des objets quand les points de repère visuels étaient enlevés. Les auteurs suggèrent que chez les poissons vivant en eau rapide, comme l'omble de fontaine, la ligne latérale peut servir de capteur de courant ou de variations de pression leur permettant de demeurer au même endroit en dépensant un minimum d'énergie.

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THE proposed functions mediated by the lateral line of fish include schooling, detection of surface and submerged prey and predators, mechanoreceptive feedback for the motor control of swimming movements, chemoreception, electroreception, and rheotactic orientation (Shaw 1969; Dijkgraaf 1952; Suga 1967; Katsuki et al. 1969; Rosen 1959; Breder 1965; Roberts 1972). It is generally concluded, regardless of the behavior(s) mediated, that lateral line sense organs of most fish respond to water movements and/or low frequency pressure differences external to the fish, resulting in fluid and cupula displacement within the canal.

In a frequently solitary species such as the brook trout (*Salvelinus fontinalis*) that lives in a "noisy" environment of rapid water flow with much turbulence created by submerged rocks, it is doubtful that many of the behavioral functions of the lateral line mentioned previously would be especially useful. Using a stream channel and swimming tube, the role of the lateral line in detecting flow discontinuities created by obstacles in the stream was examined under a variety of conditions.

Materials and Methods

Initial observation of the behavior of brook trout was conducted in a large, indoor, artificial stream channel containing a varied bottom substrate (gravel, rocks, boulders, and submerged tree branches). During the period of observations the channel had

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been in operation for several months and somewhat resembled a natural stream bed.

The stream channel was built as a rectangular flume with rounded corners with the water driven by a variable speed motor and propeller. The rectangular flume was 2.1 × 13.6 m with the average cross-sectional dimension of the stream channel 70 cm deep × 1.0 m wide. Fifty trout were placed in the channel and their stream holding positions relative to different types of bottom strata were observed through viewing windows in the sides of flume. Water velocity 0.5 m off bottom was 50 cm/s.

Position holding relative to selected geometric shapes was conducted in a Blaska-type swimming respirometer (110 cm long × 12 cm diam.) at water velocity of 54 cm/s. The objects were constructed of opaque plastic and either attached to the bottom of the swimming tube or propped between the walls by forced fit. Following a 10-h rest period, swimming velocity was gradually increased in increments of 4.5 cm/s until a velocity of 54 cm/s was reached. Swimming position was periodically observed for at least 1 h. Seven different cylinders of decreasing diameter were inserted in the respirometer and the swimming position of the fish relative to the object (degree of entrainment) was observed.

The swimming tube was also used in determining swimming endurance and was situated in a light-proof room. Fish were left at least 10 h in the tube at reduced flow rate before experiments were initiated. Velocity in the tube during swimming endurance trials was increased stepwise in increments of 4.5 cm/s and each velocity was maintained for 15 min. The entrainment object during swimming endurance trials was a 4-cm diam hemisphere attached to the floor of the swimming tube. The position of the nose of the fish was determined every 10 s with reference to 2-cm graduations on the swimming tube. In lighted condition, a closed circuit television camera and monitor were used; when such experiments were conducted in darkness, swimming position was determined by means of an infrared viewer and light source (Find-R-Scope, FWJ Industries, Mt. Prospect, Ill., USA). An electric shock screen in the back of the tube discouraged fish from resting against the screen. Terminal velocity was designated as the velocity at which the fish remained against the shock screen for 1 min, providing they had been swimming at this velocity for at least 7.5 min. If fatigue occurred sooner than this, the next lowest velocity was used as terminal velocity.

The posterior lateral line system was uni- or bilaterally denervated, following anesthetization, by making a small incision behind the cleithrum, hooking the nerve out and pulling the entire posterior portion of the nerve from its canal, and cutting it off. Examination of some initial attempts to simply transect the nerve revealed that often the nerve was not completely severed; thus, complete removal of the nerve followed by transection was adopted as a more reliable procedure. Sham operations were performed by making only the incision.

All experiments were conducted at 10 C on 10 C acclimated hatchery-reared brook trout that had been accustomed to swimming in circular tanks at a current velocity of 20 cm/s. The length of trout was 17.2 ± SD 3.4 cm. Fish were allowed 12–24 h post-operative recovery in the swimming tube before swimming tests were conducted.

Results

Although the observations in the stream channel were subjective, it was immediately apparent that the trout preferred several specific locations. Many oriented upstream behind rocks (1–3 cm diam) and remained there with no contact with the bottom and with no regular or rhythmic swimming movements. Small compensatory or damped oscillatory adjustments occurred. For larger rocks (5–20 cm diam) the fish exhibited a similar behavior, but their position was to one side or the other behind the rock. For large boulders (40+ cm diam), many trout located themselves in front of the boulder. Many other specific locations were consistently chosen by individuals from different groups of fish, but bottom strata were often so complicated that no generalizations can be made. Trout would often leave their preferred "hydrodynamic node," swim out to seize a piece of drifting debris, and upon returning, lock immediately into the same position.

Different geometric shapes were then inserted in the swimming tube where better control of experimental conditions could be maintained (Fig. 1). The entrainment position of the trout was then determined. Under lighted conditions the minimum diameter rod-shaped object on which the trout were able to entrain was 2–4 mm (Fig. 2).

Placing an object on the bottom of the swimming tube had a marked influence on swimming location (Fig. 3A and B). This behavior markedly elevated terminal velocity (Table 1A). Although not shown in Fig. 3B, the positions of fish at velocities from 45 cm/s to 80 cm/s are similar to those shown at 40.5 cm/s. Although the terminal velocity of entrained fish was twice that of fish in free stream conditions, the actual power expended by the entrained fish was significantly less than half for an unentrained fish at a given free stream velocity, as the power expended varies as the cube of the velocity (Smit et al. 1971).

Bilateral elimination of the lateral line had no influence on the terminal velocity of fish swimming in the light (Table 1A). In complete darkness, however, the terminal velocity of lateral line impaired fish was much reduced. Anesthetization and surgical trauma probably do not explain the reduced performance of the sensory-deprived fish in the dark (Table 1a). The limited ability of fish with lateral line removed to entrain on the object in darkness is apparent (Fig. 3B and C). Any instances of these fish swimming behind the

obstacle occurred when the nose of the fish was pressed directly against the object. Intact fish in the dark swam behind and slightly to the side of the object without touching it, and their degree of entrainment closely resembled the distribution shown in Fig. 3B, but was not so exact at lower velocities.

Although no difference in terminal velocity was apparent between intact and bilaterally impaired fish swimming in the light (Table 1A), unilateral deprivation induced a lateral bias concerning which side of the object the fish chose to swim on (Table 1B).

Discussion

The majority of geometric shapes used during this study were 3 cm in diameter, and the range of water velocities used was from 18 to 90 cm/s. The flow pattern downstream of an obstacle depends on the Reynolds number (R_d) (calculated by $R_d = \frac{DV}{\sqrt{}}$, where R_d = Reynolds number base on D, D = diameter of rod or sphere, V = velocity, and $\sqrt{}$ = kinematic viscosity of water at 10 C. For a sphere or cylinder of this diameter, throughout this velocity range, R_d increases from 4.1×10^3 to 2.1×10^4. According to Hoerner (1958) for spheres and rods, the stationary countercurrent circulation behind the object at R_d below 50 is well disrupted and at a Reynolds number of 10^3, vortex packages are carried away in an irregular fashion. At R_d of 10^4–10^5 a dominant frequency of alternate vortex formation is maintained with successive vortices rotating in opposite directions.

Two factors complicate any interpretation of the fishes' behavior as relating to this sequence of flow patterns. Flow in such respirometers is usually described as microturbulent and the presence of a fish behind the obstacle could modify considerably any pattern of vortex formation. Further complications exist due to wall effects that restrict normal expansion of the wake downstream. However, both the swimming position and terminal velocity indicate that the fish is using the object to its own hydrodynamic ad-

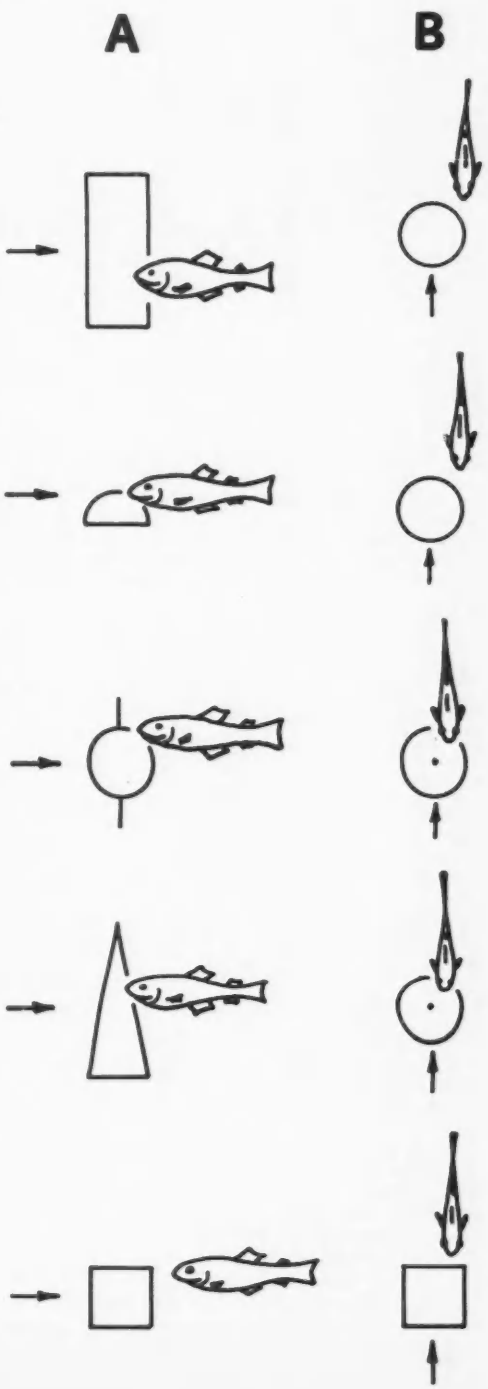

FIG. 1. Position of brook trout (*Salvelinus fontinalis*) in swimming tube as related to geometric shape of flow obstruction. A and B. Side and top view, respectively. Cross-sectional diameter of the base of objects 1–4 was 3 cm. Height of objects 1 and 4 was 12 cm. Object 5 was $3 \times 3 \times 3$ cm. The tube velocity during these observations was 54 cm/s. Although fish are shown swimming to the right of the object, swimming to the left occurred equally often.

TABLE 1. The effects of sensory impairment on terminal velocity.

A.	Light: No obstacle	Light: obstacle[a]	Dark: obstacle
Intact fish	(n = 6) 47.2 cm/±4.5 S	(4) 86.2±7.7	(4) 81.0±3.7
Bilateral removal of lateral line		(5) 88.2±5.1	(10) 50.8±18.4
Sham operated			(6) 81.7±8.7

B.		% of time and side[b]		
	Side ablated	Right	Left	No entrainment
	Left	75	0	25
	L	91	0	9
	L	89	3	8
	L	64	6	30
	Right	90	5	0
	R	0	82	18
	R	0	77	23
	R	10	74	16

[a]Hemisphere 4 cm diam.
[b]Velocity was increased stepwise, but the swimming period at each velocity was 5 min unlike experiments in A in which 15 min was used. If the fish was swimming behind and to the right of the obstacle, this is designated on the right side.

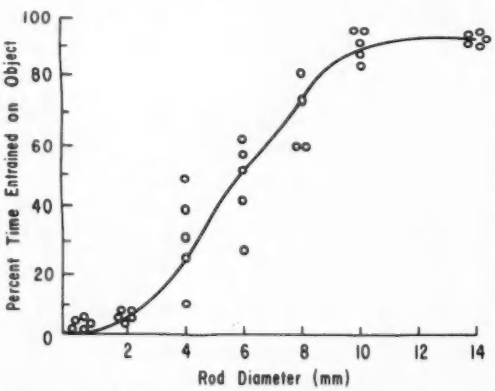

FIG. 2. Influence of rod diameter on entrainment. Five different trout were used for each trial — 35 trout in all. Swimming period was 15 min at velocity of 54 cm/s. Position of fish in tube was noted every 10 s. Experiments were conducted in the light.

vantage; also, the precision to which this entrainment occurs gives one the impression that a highly efficient control system involves sensory feedback. Under conditions of light the visual system appears to be the most likely sensory channel to provide the fish with a reference point as an indicator of body displacement. Also, the inner ear probably contributes in detecting absolute attitude with respect to gravitational forces and transients in angular and linear acceleration, permitting compensatory adjustments for yaw, pitch, and linear displacement. The tactile sensory systems (free nerve ending in fins, body, etc.) are less likely to be involved as fish in light seldom made bodily contact with the bottom or with the object. Despite the degree to which these sensory systems are involved, some measure of instantaneous work output must be considered by the neuromuscular system in order for fish to use flow discontinuities to advantage.

Under conditions of darkness, the only sensory channel impaired is vision. Possibly the tactile systems might be employed as an indicator of displacement. This cannot be disregarded during this study because visualization of swimming fish with infrared light could not accurately discern all fish contacts with the bottom or object. In darkness, intact fish remained off the bottom, behind, and to the side of the object, while lateral line impaired fish seemed to resort to swimming on the bottom with their nose often in contact with the object. The fact that lateral line impairment reduced the degree of entrainment (Fig. 3) as well as the terminal velocity (Table 1A) under conditions of darkness and induced a lateral bias in unilaterally impaired fish swimming in the light (Table 1B) suggests that this sensory channel is involved in detecting flow or pressure discontinuities, and in darkness is responsible for a considerable energy savings for the fish. This is not to say that the other sensory systems men-

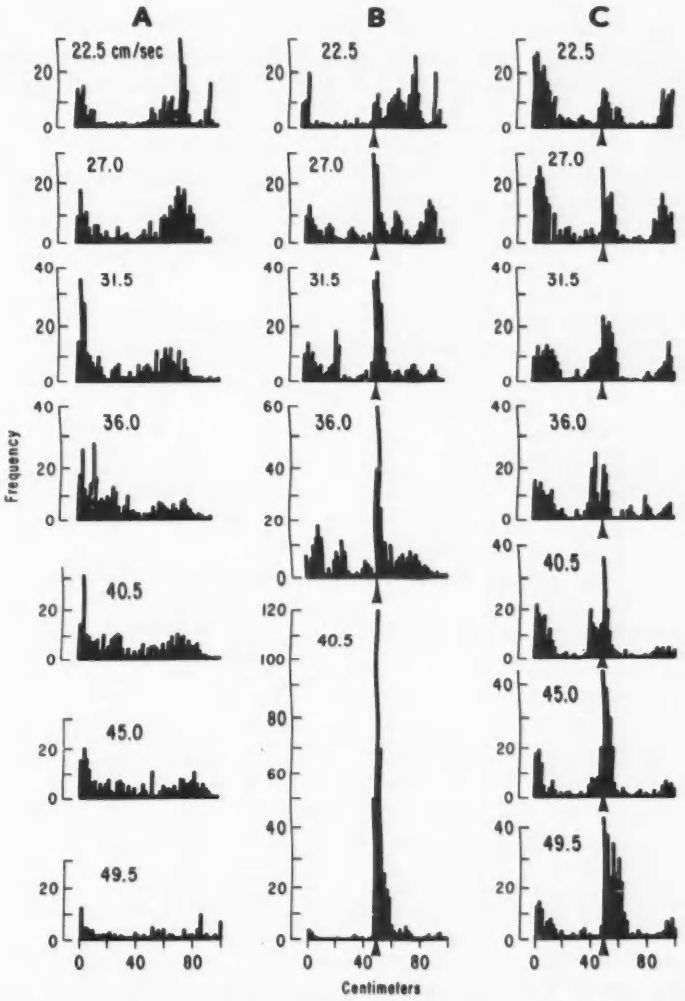

FIG. 3. Position of fish in swimming tube under various conditions. Each bin of histograms represents the position of the nose of the fish along the length of the swimming tube determined every 10 s. The histograms are the cumulative observations of four fish subjected to each of the conditions, A, B, and C. Each of the velocity increments was maintained for 15 min. A, Intact fish, light, no obstacle; B, Intact fish, light, obstacle. Arrows indicate position of obstacle — half sphere 3 cm diam; C, Bilateral ablation of lateral line, dark, obstacle.

tioned above are not involved. If some sort of standing wave expressed as a discontinuity in flow and/or pressure is created by an obstacle, then the posterior lateral line system would seem like a very appropriate system to detect such a configuration. The hair cells would not necessarily have to respond tonically or have an exceptionally low frequency response but might be effective in detecting abrupt changes in flow pattern around the fish. As with most studies on sensory deprivation, including those that are sham controlled, it may be argued that cutting a channel of impor-

tant sensory input, however its function may be related to the test task, could result in a general sensory imbalance and reduced performance. However, the fact that denervation did not affect swimming performance under lighted conditions provides some evidence that the lateral line is probably not functioning in locomotive reflexes as an external proprioceptor monitoring body flections. It would have been interesting to test fish 1 wk after denervation rather than 12–24 h as a means of examining the permanency of the impaired entrainment; however, this was not done.

One might have expected the anterior portion of the lateral line with its numerous pit and canal organs to supply sufficient cues for entrainment to occur. The capacity of the anterior system apparently was not sufficient to compensate for denervation of the posterior system, possibly because information from the anterior may be disoriented in the absence of posterior input.

Dijkgraaf (1962) emphasized the "damming phenomenon" that is created by a moving object in stationary water causing pressure (Standruch) and displacement (Aufstau) disturbances that are important stimuli for the lateral line. What we propose here for the brook trout is very similar except the object is stationary and the water is moving. This does somewhat change the emphasis from a moving prey or predator to a stationary rock but the principles are the same. Dijkgraaf (1934) demonstrated the importance of the lateral line in rheotactic orientation to discontinuities in water currents but very logically favored visual orientation under continuous light.

Osborne (1961) clearly recognized the importance of ascending salmon's utilizing flow discontinuities to reduce their energy expenditure by means of the Betz-Knoller effect (Kuchemann and Weber 1953) and concluded from an engineering standpoint that the ability of salmon to ascend such great distances with so little fuel consumption presents a near paradox. Possibly the lateral line may be involved in determining such gradients in stream velocity.

Brook trout were able to entrain on a vertical rod of 3 mm diam, and this is probably not surprising to some investigators, as it is commonly observed that collection of a small piece of debris on the forward screen of swimming respirometer provides sufficient stimulus for the fish to entrain on. As our experiments were done under lighted conditions, we cannot say that a 3-mm object would be as effective in the dark.

Projections to the cerebellum from the lateral line seem similar in many respects to other sensory receptors of the acoustical–equilibrium system in fish and their functional role in representing the animals' orientation at any given moment may be comparable (Peterson 1971; Alneas 1973).

Acknowledgments

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Method of Determining Natural Mortality in the Northern Fur Seal (*Callorhinus ursinus*) from Known Pups and Kill by Age and Sex

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LANDER, R. H. 1975. Method of determining natural mortality in the northern fur seal (*Callorhinus ursinus*) from known pups and kill by age and sex. J. Fish. Res. Board Can. 32: 2447–2452.

From known values of the number of northern fur seals, *Callorhinus ursinus*, born in a given year and the commercial kill of older animals of this species, by age and sex, a method is given for approximating natural mortality or survival 1) between times of birth and the first kill and 2) during the successive ages of kill. An example with hypothetical data indicates small relative error in these estimators, although bias may be considerable in certain of the associated age-specific estimators of abundance and exploitation rates. The problem of bias warrants further investigation. Estimates of natural survival from birth to the start of the kill at age 2 yr agree closely with independent tagging estimates for the 1964–66 year-classes of males from the Pribilof Islands, Alaska. For this stock, further applications of the present method to the 1961–66 year-classes of males indicate natural survival of 31–42% during ages 0–2 yr and 84–89% annually during ages 2–5 yr.

LANDER, R. H. 1975. Method of determining natural mortality in the northern fur seal (*Callorhinus ursinus*) from known pups and kill by age and sex. J. Fish. Res. Board Can. 32: 2447–2452.

A partir de données connues sur le nombre de phoques de Pribilof, *Callorhinus ursinus*, qui naissent une année donnée et de celui, par âge et par sexe, des vieux animaux abattus par la chasse commerciale, nous décrivons une méthode d'estimation de mortalité ou de survie naturelles 1) entre la naissance et le début de l'abattage et 2) aux âges successifs d'abattage. Un exemple hypothétique indique une faible erreur relative dans ces paramètres d'estimation, bien que l'erreur systématique puisse être considérable dans certains paramètres associés spécifiques à l'âge, lorsqu'il s'agit d'estimer l'abondance et le taux d'exploitation. Le problème de l'erreur systématique devra être étudié davantage. Les estimés préliminaires de la survie naturelle entre la naissance et le début de l'abattage à l'âge de 2 ans sont en accord étroit avec les estimés obtenus indépendamment par des marquages de mâles des classes annuelles de 1964–66 aux îles Pribilof, Alaska. Pour ce stock, d'autres applications de la présente méthode aux mâles des classes d'âge de 1961–66 indiquent une survie naturelle de 31–42% entre 0 et 2 ans d'âge et de 84–89% entre 2 et 5 ans d'âge.

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THE primary management objective of the North Pacific Fur Seal Commission — whose member nations are Canada, Japan, the Soviet Union, and the United States — is to harvest the northern fur seal (*Callorhinus ursinus*) on the basis of maximum sustainable yield (North Pacific Fur Seal Commission 1962). To this end, population research for determining the status of stocks has included tagging–recovery experiments to approximate abundance, natural mortality, and rate of exploitation (Kenyon et al. 1954; Chapman, 1961, 1964, 1973; North Pacific Fur Seal Commission 1962, 1969, 1971). In addition to the usual problem of tag loss, two other difficulties arise. First is applying enough tags to different age-groups of a year-class to ensure reliable estimates of age-specific natural mortality. Second is the fact that tagging–recovery estimates of abundance vary with age at recovery and, in the case of newborn pups, are biased upward even when corrected for tag loss (Roppel et al. 1966).

I present a method of determining natural mortaltiy which does not require tagging. Two estimates are involved: total mortality from birth to the start of the first kill and average annual mortality during the several ages of kill. For any year-class the required data are counts or estimates of 1) the number of pups born and 2) the kill by sex and age. The method is then applied to data

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from the 1961–66 year-classes of males from the Pribilof Islands, Alaska.

Method of Estimation

The method takes advantage of 1) the strong tendency of northern fur seals to return to their area of birth and 2) the fact that ages 3–4 yr account for most of the total kill (North Pacific Fur Seal Commission 1962, 1969, 1971, 1975). Limits for certain parameters are developed initially; then the estimating scheme is illustrated by assigning a set of hypothetical parameters, generating the type of data observed in practice, and comparing the resulting estimates with the assigned values. Applications are in the next section. The method is a modification of the limit-mean model for approximating ocean mortality, maturity, and abundance of multireturn species of salmon (Lander 1973; Lander and Henry 1973).

Notation and Assumptions

The notation below refers to male animals of a specified year-class:

- N_o = number of pups born
- s^* = proportion of N_o that survive natural mortality until the start of the kill (about July 1) at age 2 yr
- s = proportion of $N_o s^*$ that survive natural mortality each year from the end of the kill (about July 31) at age 2 yr until the start of the kill at age 5 yr
- i = subscript designating age in years (i = 2, 3, 4, 5)
- R_i = number of animals alive at the start of the harvest at age i
- K_i = number killed at age i
- N_i = number of survivors when the kill at age i ends
- u_i = age specific exploitation rate
- u_T = total exploitation rate of the year-class = $\Sigma K_i/R_2$
- M,*M = average monthly rates of natural mortality associated with s^* and s, respectively
- M_T = average monthly rate of natural mortality from birth to the start of the kill at age 5 yr
- L, U = subscripts, respectively, denoting a lower or upper limit of a parameter

The assumptions are as follows:

1) $M^* \geqslant M_T \geqslant M$, i.e. $M^*_L = M_{TU}$ and $M_{TL} = M_U$.

2) $M = 0$ during the kill at any age i (very few young males are found dead on the Pribilof Islands during any of the several months they are on land); the annual natural mortality fraction, $1 - s$, is attributed to the 11 mo the seals are not harvested.

3) $R_{5L} = K_5$.

4) $R_{5U} = K_4 + K_5$. An upper bound for R_5 depends on the operational plan under which a portion of the males are left to form the breeding reserve. One approach is to estimate this escapement from the number of adult males as attempted by Chapman (1964). A simpler approach is to express the bound in terms of the kill parameters. As a referee has pointed out, $R_5 \leqslant K_4 + K_5$ is equivalent to $R_5 \leqslant R_5u_5 + R_4u_4$, i.e. to $R_5(1 - u_5) < R_4u_4$. Since $R_5 = (R_4 - K_4)s = (R_4 - R_4u_4)s = R_4(1 - u_4)s$, the inequality reduces to $R_4(1 - u_5)(1 - u_4)s \leqslant u_4R_4$ or $(1 - u_4)(1 - u_5)s \leqslant u_4$. Regardless of u_5 and s, this holds if $u_4 \geqslant .50$. The exploitation rate on the 4-yr age-class under the present management regime has in fact exceeded 0.50 (e.g. Marine Mammal Biological Laboratory, 1972), so that this is a reasonable assumption *under present exploitation policies.*

5) s is constant during ages 2–5 yr.

The kill schedule is given below:

$$K_2 = u_2R_2 = u_2N_os^* \quad (1)$$

$$K_3 = u_3R_3 = u_3N_os^*s(1 - u_2) \quad (2)$$

$$K_4 = u_4R_4 = u_4N_os^*s^2(1 - u_2)(1 - u_3) \quad (3)$$

$$K_5 = u_5R_5 = u_5N_os^*s^3(1 - u_2)(1 - u_3)(1 - u_4) \quad (4)$$

The schedules of returns and postkill survivors are related by:

$$N_i = R_i - K_i = R_i(1 - u_i) \quad (5)$$

Starting with Equation 1, solutions for the K_i/N_o can be substituted into Equations 2–4. After collecting terms and rearranging, Equation 4 becomes:

$$K_5/N_o = u_5(R_5/N_o) = u_5[s^*s^3 - (K_2/N_o)s^3 - (K_3/N_o)s^2 - (K_4/N_o)s] \quad (6)$$

Dividing the first and last expression in Equation 6 by u_5 and rearranging:

$$s^* = K_2/N_o + (K_3/N_o)s^{-1} + (K_4/N_o)s^{-2} + (K_5/N_o)s^{-3}(u_5)^{-1} \quad (7)$$

The survival proportions and monthly rates of instantaneous natural mortality are related as follows:

$$M^* = (-\ln s^*)/24 \quad (8)$$

$$M = (-\ln s)/11 \quad (9)$$

$$M_T = (-\ln s^*s^3)/57 = (24\,M^* + 33M)/57 \quad (10)$$

From Assumptions 3–4, limits for s^* can be written from Equation 7:

$$s^*_L = K_2/N_o + (K_3/N_o)s^{-1} + (K_4/N_o)s^{-2} + (K_5/N_o)s^{-3} \quad (11)$$

$$s^*_U = K_2/N_o + (K_3/N_o)s^{-1} + (K_4/N_o)s^{-2} + [(K_4 + K_5)/N_o]s^{-3} \quad (12)$$

One may start with $s = 1.0$ ($M = 0$), insert decreasing trial values of s in Equations 11–12 then apply Equations 8–10 in order to find associated values of (increasing) M, (decreasing) M^*_L and M^*_U, and (increasing) M_{TL} and M_{TU}. The last trial is when $M^*_L = M_{TU}$ (Assumption 1), i.e. when $s = s_L$ and $M = M_U$.

Given s_L as above, taking $s_U = 1.0$, and recalling $R_{5U} = K_4 + K_5$ and $R_{5L} = K_5$, one can work backward in time to construct limits on the survivor schedules, R_i and N_i, as follows:

Lower limits	Upper limits
$R_{5L} = K_5$	$R_{5U} = K_4 + K_5$
$N_{4L} = K_5$	$N_{4U} = R_{5U}/s_L$
$R_{4L} = K_4 + K_5$	$R_{4U} = N_{4U} + K_4$
$N_{3L} = K_4 + K_5$	$N_{3U} = R_{4U}/s_L$
$R_{3L} = K_3 + K_4 + K_5$	$R_{3U} = N_{3U} + K_3$
$N_{2L} = K_3 + K_4 + K_5$	$N_{2U} = R_{3U}/s_L$
$R_{2L} = K_2 + K_3 + K_4 + K_5 = \Sigma K_i$	$R_{2U} = N_{2U} + K_2$

The upper limits account for natural deaths while the lower limits do not. We shall find when the method is applied that about 90% of the kill is at ages 3–4 yr: when the foregoing limits are graphed, the distances are relatively small between R_{3L} and N_{3U} as they are between R_{4L} and N_{4U}. This fact can be utilized to estimate the target parameter, s. In particular, the size of the year-class in mid-July is estimated at age 3 yr as the mean of R_{3L} and N_{3U} and at age 4 yr as the mean of R_{4L} and N_{4U}. Then $K_3/2$ is subtracted from the estimate at age 3 yr, and $K_4/2$ is added to the estimate at age 4 yr, to estimate N_3 and R_4, respectively. Their ratio is the estimator of s. To summarize after substitution of the pertinent limits from the above survivor schedules:

$$\hat{s} = \hat{R}_4/\hat{N}_3 = \frac{K_4 + (K_4 + K_5)(1 + s_L^{-1})}{K_4 s_L^{-1} + (K_4 + K_5)(1 + s_L^{-2})} \quad (13)$$

Then from Equations 10 and 14:

$$\hat{M} = (-\ln s)/11 \quad (14)$$

The value of $\hat{M}$, along with the limits for R_5, may be used to find M_{TL} and M_{TU} from trial solutions of Equation 7 (first multiplying by s^3 and rearranging) in its exponential form:

$$e^{-57M_{TL}} - (K_2/N_o)e^{-33\hat{M}} - (K_3/N_o)e^{-22\hat{M}} - (K_4/N_o)e^{-11\hat{M}} - (K_4 + K_5)/N_o = 0 \quad (15)$$

$$e^{-57M_{TU}} - (K_2/N_o)e^{-33\hat{M}} - (K_3/N_o)e^{-22\hat{M}} - (K_4/N_o)e^{-11\hat{M}} - K_5/N_o = 0 \quad (16)$$

The estimator of M_T is then taken to be the mean of its limits:

$$\hat{M}_T = (M_{TL} + M_{TU})/2 \quad (17)$$

Given $\hat{M}$ and $\hat{M}_T$, M^* is estimated from Equation 10:

$$\hat{M}^* = (57\hat{M}_T - 33\hat{M})/24 \quad (18)$$

Then from Equations 8 and 18:

$$s^* = e^{-24\hat{M}^*} \quad (19)$$

Equations 14–19 show explicitly the same result as if $\hat{s}$ (Equation 13) were used in the scheme for finding s_L: if $\hat{s}$ (instead of a trial value of s) is inserted in Equations 11–12 and Equations 8 and 10 are applied, then the means of the limits for M_T and M^* are identical to the respective estimators in Equations 17–18.

Finally, u_T and the u_i can be estimated from Equations 1–4, 13 and 18:

$$\hat{u}_T = \Sigma K_i/\hat{R}_2 = \Sigma K_i[N_o\hat{s}^*]^{-1} \quad (20)$$

$$\hat{u}_2 = (K_2/N_o)(\hat{s}^*)^{-1} \quad (21)$$

$$\hat{u}_3 = (K_3/K_2)\hat{u}_2[\hat{s}(1 - \hat{u}_2)]^{-1} \quad (22)$$

$$\hat{u}_4 = (K_4/K_3)\hat{u}_3[\hat{s}(1 - \hat{u}_3)]^{-1} \quad (23)$$

$$\hat{u}_5 = (K_5/K_4)\hat{u}_4[\hat{s}(1 - \hat{u}_4)]^{-1} \quad (24)$$

Example with Hypothetical Data

Table 1 gives a set of hypothetical parameters, data as observed in practice (N_o and K_i), and selected results from indicated text equations (the schedules and limits include fractions of one seal for the sake of numerical completeness). The relative error or bias in this example is quite small for the estimates of s^* and s in Table 1: the values of [(estimate–parameter value)/(parameter value)] × 100 are + 1.4% for $\hat{s}^*$ and + 3.6% for $\hat{s}$ (additional calculations in progress indicate that the bias in these two parameter estimates will be small in general, usually less than 10%, for most observed values of K_i/N_o).

Figure 1 shows from Table 1 the limits for the survivor schedule, with certain checkpoints identified, to illustrate the estimation of s (Equation 13). Heavy solid lines bound the unknown survivor schedule (dotted line). Points at mid-July on the age axis are at the means of R_{3L}, N_{3U} and of R_{4L}, N_{4U}. Light solid lines with arrows show 1) time displacements to "x" marks (N_3 and R_4 which are not labeled) and 2) their connection ($\hat{s}$) as contrasted with unknown s. The slope difference is small. $\hat{R}_4$ and $\hat{N}_3$ contain considerable error individually (e.g. if $\hat{R}_4$ is used along with $\hat{s}$ and K_4 to estimate R_5, the result can be shown from values in Table 1 to be 76% too large).

The main points to be emphasized for the method are 1) the relatively small bias in estimating s^* and s and 2) the fact it can be extended to any age and sex distribution of known kills provided the pup population by sex is known also, or a 50:50 sex ratio in a known total of newborn pups is assumed.

Application to 1961–66 Year-Classes

Table 2 gives the basic data (N_o and K_i) for the 1961–66 year-classes on the Pribilof Islands. Table 3 gives values of s_L along with estimates of s^* and s. Estimates for the other parameters are not included: values in Table 1 indicate, for example, an increasing bias in the $\hat{u}_i$ as one proceeds from age 2 to age 5 yr. The data in Table 2 show that $K_3 + K_4$ account for 91% of ΣK_i on the average (range 87–93%). These high contributions at ages 3–4 yr are an important basis of the method as noted earlier.

Comparison with tagging results indicates high consistency with respect to the $\hat{s}^*$. Table 11 of Marine Mammal Biological Laboratory (1972) gives the following estimates of natural survival for ages 0–1 and 1–2 yr, respectively, in the indicated year-classes: 1964 — 0.68, 0.63; 1965 —

TABLE 1. — Example with hypothetical data to illustrate method of estimating natural mortality and related parameters for male northern fur seals (*Callorhinus ursinus*) as described in text.

Item and value	Text equation	Limit or estimate	Text equation
Input parameters	—	$s_L = 0.769562$	8–12
$N_o = 100{,}000$	—	$\hat{s} = 0.828579$	13
$s^* = 0.30$	—	$\hat{M} = 0.017095$/mo	14
$s = 0.80$	—	$M_{TL} = 0.027555$/mo	15 or 8–12
$u_2 = 0.05$	—	$M_{TU} = 0.033990$/mo	16 or 8–12
$u_3 = 0.50$	—	$\hat{M}_T = 0.030773$/mo	17
$u_4 = 0.70$	—	$M^*_L = 0.041938$/mo	8–12
$u_5 = 0.20$	—	$M^*_U = 0.057221$/mo	8–12
$M^* = 0.050166$/mo	9	$\hat{M}^* = 0.049580$/mo	18
$M = 0.020286$/mo	10	$\hat{s}^* = 0.304246$	19
$M_T = 0.032867$/mo	11	$\hat{u}_T = 0.648203$	20
Kill schedule	1–5	$\hat{u}_2 = 0.049302$	21
$K_2 = 1{,}500$	—	$\hat{u}_3 = 0.475662$	22
$K_3 = 11{,}400$	—	$\hat{u}_4 = 0.613114$	23
$K_4 = 6{,}384$	—	$\hat{u}_5 = 0.131150$	24
$K_5 = 437.7600$	—		—
$\Sigma K_i = 19{,}721.7600$	—		—
Survivor schedule	1–5	Limits for R_i and N_i	1–5
$R_2 = 30{,}000$	—	Lower Upper	—
$N_2 = 28{,}500$	—	R_5 437.7600 6,821.7600	—
$R_3 = 22{,}800$	—	N_4 437.7600 8,864.4710	—
$N_3 = 11{,}400$	—	R_4 6,821.7600 15,248.4710	—
$R_4 = 9{,}120$	—	N_3 6,821.7600 19,814.4801	—
$N_4 = 2{,}736$	—	R_3 18,221.7600 31,214.4801	—
$R_5 = 2{,}188.8000$	—	N_2 18,221.7600 40,561.3584	—
$N_5 = 1{,}751.0400$	—	R_2 19,721.7600 42,061.3584	—

TABLE 2. Estimated number of male pups born and kill of males by age on the Pribilof Islands, Alaska, 1961–66 year-classes (Marine Mammal Division, 1973).

Year-class	Number of male pups born (N_o)	Kill of males by age (K_i)					
		K_2	K_3	K_4	K_5	ΣK_i	$\Sigma K_i/N_o$
1961	219,000	2,019	29,416	14,638	1,772	47,845	0.2185
1962	181,000	3,678	22,745	16,037	1,679	44,139	0.2439
1963	171,500	1,431	31,121	15,523	1,948	50,023	0.2917
1964	185,000	3,534	34,613	16,959	2,149	57,255	0.3095
1965	173,500	2,940	23,149	12,769	1,278	40,136	0.2313
1966	194,000	2,116	20,471	13,822	1,805	38,214	0.1970

0.59, 0.59; and 1966 — 0.38, 0.84. The products of these paired tagging estimates (i.e. the total natural survival during ages 0–2 years) for the respective year-classes are 0.43, 0.35, and 0.32. Corresponding estimates of s^* in Table 3 for the 1964–66 year-classes are 0.42, 0.34, and 0.31. The estimates differ by only 1% in each case.

Values of s^* in Table 3 suggest an increasing trend of natural survival during ages 0–2 yr for the 1961–64 year-classes (33–42%), then a rather abrupt decline to 34% for the 1965 year-class and 31% for the 1966 year-class. An understanding of the biological mechanisms causing fluctuations in early natural survival is desirable for rational management of the fur seal herds on the Pribilof Islands and on rookeries of the USSR.

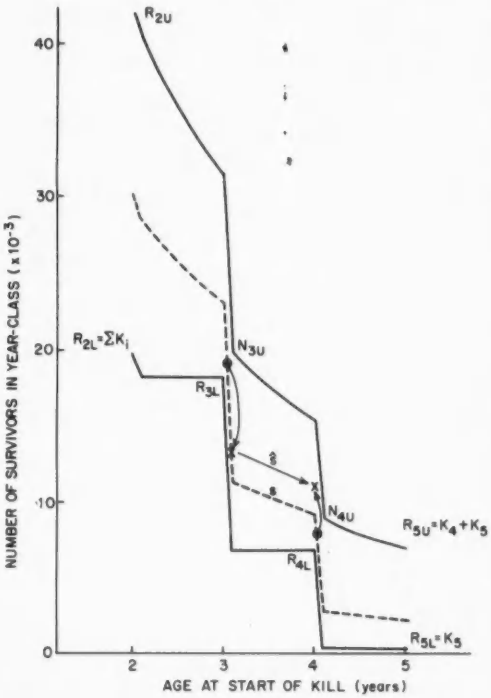

FIG. 1. Limits (heavy solid lines) and checkpoints for the unknown survivor schedule (dashed line) in example (Table 1). Light solid lines with arrows show sequence for $\hat{s}$, compared with unknown s, as described in the text and Equation 13.

TABLE 3. Values of s_L and estimates of total natural survival during ages 0–2 yr ($\hat{s}^*$) and of annual natural survival during ages 2–5 yr ($\hat{s}$) for the 1961–66 year-classes of male fur seals from the Pribilof Islands, Alaska.

Year-class	s_L	$\hat{s}^*$	$\hat{s}$
1961	.783	0.329	0.840
1962	.820	0.357	0.870
1963	.837	0.406	0.884
1964	.843	0.421	0.888
1965	.796	0.342	0.851
1966	.783	0.307	0.840

Discussion

Tagging–recovery experiments have been used in the past to estimate age-specific abundance, natural mortality, and rate of exploitation in fur seals. It is difficult to apply and recover enough tags from different age-groups of a year-class to ensure reliable estimates. Furthermore, the estimated abundance of a given age-group (say pups or yearlings) may vary with age of recovery even after corrections are made for tag loss and, in the case of animals older than pups, for uncertainty in age at the time of tagging. The present method is an effort to avoid some of the difficulty with tagging–recovery experiments, but is not intended to replace them entirely. In fact, Assumption 4 is based on evidence from tagging–recovery and is a key assumption.

Several sets of unknown parameters (post-pup abundance, rates of natural mortality, and exploitation) may give rise to the data (number of pups and kill by age) observed from a year-class: the problem of estimation has no unique solution. The present approach is to devise upper and lower limits on the survivor schedule; the resulting method depends on the relatively high proportion of male seals harvested at ages 3–4 yr on the Pribilof Islands, Alaska. Trial calculations (e.g. Table 1) indicate bias associated with the method will in general be small for estimates of natural mortality 1) from birth to the start of the kill at age 2 yr and 2) as averaged annually for the several ages of harvest. Bias in estimates of associated parameters, on the other hand, may increase sharply as one proceeds from the youngest to the oldest age of kill. Until the problem of bias is more fully investigated, therefore, application should be limited to estimates of s^*, s, R_2, N_2, u_2, and u_T.

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Pleomerism, the Widespread Tendency Among Related Fish Species for Vertebral Number to be Correlated with Maximum Body Length

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LINDSEY, C. C. 1975. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J. Fish. Res. Board Can. 32: 2453–2469.

Data for 3137 fish species were analyzed for possible correlations, among related species, between mean vertebral number (V) and maximum recorded body length (L). Of 118 families having counts for over 4 species, 90 show a positive correlation; statistically significant correlations are positive in 45 and negative in only 2 families. The relationship can be expressed by the power function $V = CL^m$, in which C and m are constants characteristic of each family or taxon. Among families with a significant correlation between V and L, the median value of m is 0.12 (representing about 10% vertebral increase for each doubling of length) but m ranges widely, from −0.033 to +0.416. Families with high mean vertebral counts tend to have high m values. The phenomenon, termed pleomerism, occurs within genera as well as within families, and sometimes between races, between populations, or even between the sexes. It exists in widely different shapes of fishes (e.g. sharks, mackerels, sea horses, sand lances, and lumpfish), and in caecilians and in sea snakes. Latitudinal gradients in vertebral number (Jordan's rule) are often reinforced by pleomerism coupled with latitudinal gradients in maximum length, but gradients in vertebral numbers persist even when effect of maximum length is removed. Although its cause is unknown, pleomerism is taxonomically useful in predicting adult sizes of species known only from young specimens, and in unmasking synonymies of "species" based on young of other named species. Vertebral number considered in conjunction with maximum size is more reliable for making taxonomic judgments at all levels than is vertebral number alone. Moreover, if the cause of pleomerism is functional, it may provide information on locomotory mechanisms and on population biology, since mean vertebral number in each population may be matched to that body size at which selection operates most significantly.

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Nous avons analysé les données recueillies sur 3137 espèces de poissons en vue d'établir des corrélations possibles, au sein d'espèces apparentées, entre le nombre moyen de vertèbres (V) et la longueur corporelle maximale (L) enrégistrée. Sur 118 familles dont les comptes vertébraux sont accessibles pour plus de 4 espèces, 90 montrent une corrélation positive; les corrélations statistiquement significatives sont positives dans 45 et négatives dans seulement 2 familles. Cette relation peut s'exprimer par l'équation $V = CL^m$, dans laquelle C et m sont des constantes caractéristiques de chaque famille ou taxon. Parmi les familles où il y a une corrélation significative entre V et L, la valeur médiane de m est de 0.12 (soit une augmentation de 10% du nombre vertébral chaque fois que la longueur est portée au double), mais m accuse de grandes variations, de −0.033 à +0.416. Les familles dont le compte vertébral moyen est élevé tendent à avoir des valeurs de m élevées. Ce phénomène, appelé pléomérisme, se rencontre à l'intérieur des genres aussi bien que des familles, et parfois entre races, populations et même sexes. Il existe chez des poissons de formes fort différentes (e.g. requins, maquereaux, hippocampes, lançons et poules de mer), chez les caeciliens et les serpents de mer. Les gradients latitudinaux du nombre de vertèbres (loi de Jordan) sont souvent renforcés par un pléomérisme couplé à des gradients latitudinaux de longueur maximale, mais les gradients latitudinaux du nombre de vertèbres persistent, même lorsque l'effet de la longueur maximale a été éliminé. Bien que la cause en soit inconnue, le pléomérisme est utile en taxonomie, en ceci qu'il permet de prédire les tailles adultes d'espèces connues seulement à partir de jeunes spécimens, et de démasquer la synonymie d'« espèces » fondée sur les jeunes d'espèces déjà nommées. Le compte vertébral, considéré conjointement avec la taille maximale, est plus sûr que le compte vertébral seul lorsqu'il s'agit de porter des jugements taxonomiques. De plus, si le pléomérisme se rapporte à la fonction, il peut

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nous renseigner sur les mécanismes locomoteurs et sur la biologie des populations, car la moyenne vertébrale de chaque population peut être appariée à cette longueur du corps à laquelle la sélection agit de la façon la plus significative.

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A remarkably widespread tendency exists among fishes and some other animals for larger species to have more vertebrae than their smaller relatives. A survey of vertebral counts in several thousand species shows the phenomenon to occur in widely different shapes and sizes of fish, and at several taxonomic levels. Bailey and Gosline (1955) observed in North American species of percid fishes a positive correlation between vertebral count and adult size; otherwise, except for several references to a correlation within more restricted groups of species, the prevalence of the phenomenon seems to have been largely unnoticed.

The demonstration of such a pervasive correlation raises intriguing questions about possible causes, but this paper is intended primarily to document the phenomenon, and does not attempt an explanation. It does explore the possibility that the well-known tendency for a clinal increase in vertebral numbers from the tropics towards the poles ("Jordan's rule") may be a reflection of this correlation between vertebrae and body length. Some of the possible applications of the phenomenon to taxonomy will be discussed. The possibility will also be raised of analyzing populations in terms of different selective pressures at different stages of the life history operating on fixed characters (such as vertebral number) which may convey different selective advantages at different body sizes.

Terminology

The term "pleomerism" will be used henceforth to refer to a correlation, among different animals, between their numbers of meristic parts and their characteristic body sizes. The taxa examined here are primarily different species within one family, but pleomerism may occur at any taxonomic level (e.g. populations or races within a species, or families within an order or a class). The meristic parts examined here are the vertebrae, but pleomerism may be sought in any meristic series whose number does not increase with age, in any animal group (e.g. scales or fin rays in fish, scutes in snakes, metameres in worms, antennal segments in insects). The "characteristic body size" used here is the maximum recorded size, but pleomerism might perhaps emerge more strongly if some other body size were examined (e.g. size at first sexual maturity, or at metamorphosis, or at hatching).

The roots of the term "pleomerism" are *pleo-* (more) and *merism* (the phenomenon of repetition of parts). "Pleomerism," referring to *number* of parts, is comparable to "allomorphosis" which refers to the relation of the *size* "of parts of organisms at some definite age to wholes or parts also at some definite age, but of different groups (races, varieties, species, genera)" (Huxley et al. 1941), or, more simply, to differences in *proportions* between adults of related groups.

The expression "Jordan's rule" needs clarification. It is used here in reference to the observation that fishes in polar or cool waters have more vertebrae or other meristic parts than have related forms in tropical or warm waters. Günther (1862:65) first noted the trend, in vertebral counts in the family Labridae. Gill (1864) showed that the trend occurs in other groups of fishes. David Starr Jordan (in 1892 and in later papers) confirmed these observations, illustrated them with more fish groups, and expanded them to include other meristic elements. The expression "Jordan's rule" (or "Jordan's law") came to be applied to the phenomenon (e.g. Gabriel 1944; Rass 1948; Seymour 1959) and so appears in the Collegiate Dictionary of Zoology (Pennak 1964).

Unfortunately the expression is sometimes used in a different context. Mayr (1963:487) wrote "D. S. Jordan emphasizes the very same point most frequently stressed by Wagner, namely, that closely related forms (species or subspecies) occur in adjacent areas. When J. A. Allen suggested in a review that this observation should be called 'Jordan's law,' Jordan (1908) modestly pointed out that he was merely reiterating what Wagner had stated 35 years earlier. In this he was right; if the law were to be named at all, which is doubtful, it should be called 'Wagner's law'."

Webster's Third New International Dictionary gives "Jordan's law" only in Allen's sense. Gray (1967) lists "Jordan's law 1" in Allen's sense, and "Jordan's law 2" as referring to vertebrae. I concur with Mayr's view, and recommend that "Jordan's rule" be used in conformity with Pennak (1964), and in the sense of Gray's "Jordan's law 2," with reference to meristic numbers and latitude.

Materials and Methods

Vertebral counts and maximum recorded body lengths were assembled for as many fish species as possible by a search of the scientific literature in a variety of libraries intermittently over 5 yr. For a few species unpublished data were provided by colleagues or from counts made by the author. The search was terminated due to diminishing returns after many of the most obvious and readily available of the world's journals and books had been searched. Additional published vertebral counts could probably be discovered in most of the major fish groups, newly published counts appear regularly, and a great many counts exist in unpublished research files. Nevertheless, the counts for the 3137 fish species (in 252 families) assembled during this study probably represent a majority of all those known counts. Data for 2651 species in 85 families are summarized in Table 1; counts are not given for the remaining species which fell in families for which sample sizes were less than 10 and which did not show a significant correlation between vertebral number and maximum length.

During library searches, data were entered separately for each species on punch cards. These were then collated, duplicates due to synonymy were removed, data were transcribed to computer cards (used also in later calculations), and a print-out was obtained arranged in phyletic sequence. For each species the following data were shown if available: family code number (using the 604 numbers assigned by Berg 1940); family name; species number (assigned arbitrarily to facilitate retrieval as cards were punched); genus name; species name; vertebral counts (mean, upper, and lower recorded counts); most northerly and most southerly latitudinal limits; habitat (marine, freshwater, or both); start and end of spawning time (year divided into 24 numbered fortnights); egg diameter in millimeters; and maximum recorded total length in centimeters (converted if necessary from standard or fork length using measurements from illustrations). Supplementary data were recorded for some species (larval size at hatching, larval size at yolk absorption, body depth, vertebral width or other dimensions, other meristic characters). Not all of these data are analyzed in the present report.

In the main analysis (Table 1) the smallest taxonomic unit considered is that of species; where separate data were published for more than one subspecies, race, or population, a mean vertebral number for the whole species was calculated. Centra in the Weberian apparatus, and the urostyle, are intended to be included, although occasional authors may have excluded the urostyle without stating so. If different authors recorded different maximum sizes for a species, the largest was selected.

In addition to the 85 families listed in Table 1, 33 others had between 5 and 9 species but no significant V-L correlation. They are omitted from further consideration.

The classification by Berg (1940) of higher fish groups has been much altered by recent authors (e.g. Greenwood et al. 1966), but his numbering of families (whose limits have been subject to less change) was followed for convenience in coding. Family names and limits used in this analysis are in most cases those of Greenwood et al.; any which vary significantly from those of Berg are indicated by footnotes on Table 1.

Analyses and graphs of body lengths and vertebral numbers use data transformed to natural logarithms; means are geometric rather than arithmetic.

Results

Vertebral Number and Maximum Body Length

Typical correlations between mean vertebral count and maximum length among related species are shown in Fig. 1 and 2. Each group displays pleomerism, i.e. the larger species tend to have more vertebrae. The relationship is conveniently represented by the power function

$$V = CL^m$$

where V is mean vertebral number for a species, L is its maximum recorded length in centimeters, and C and m are constants characteristic for the family or other taxon. The value of the exponent m is always less than unity, so that on arithmetic axes the increase in vertebral number becomes progressively less at larger sizes. Hence, all plots are presented on logarithmic axes, and the regression lines of $\log_e V$ on $\log_e L$ have been calculated whose slopes and intercept at $L = 1$ correspond to m and C, respectively.

Within families — Of the 47 families having a statistically significant correlation between V and L, 45 were positive and only 2 were negative (Table 1 and Fig. 2). In the remaining 38 families in Table 1, whose correlation coefficient was not significant, 22 were positive, 14 negative, and 2 zero. Other families with from 5 to 9 species and lacking significant correlations, which are omitted from Table 1, included 23 with r positive, 6 with r negative, and 4 with r zero. Correlations were not recorded for the remaining 134 families which were each represented by fewer than 5 species.

Evidently there is a striking tendency for vertebral number to be positively correlated with maximum length in a great many families. The extent to which individual species depart from the overall correlation within a family is attributable in part to the degree of their dissimilarity in body form. Families consisting of species having closely similar shape show less scatter about the V-L regression line than those displaying wide differences in stoutness. For example, the rather wide scatter in the family Scombridae (Fig. 1C) is

TABLE 1. Means and ranges of vertebral number (V) and maximum length (L), and statistics of the equation $V = CL^m$, for all families with $n > 9$ or with significant r.

Family No.[e]	Name	No. of species	Vertebrae Mean[f]	Vertebrae Range[g]	Max. length (*cm*) Mean[f]	Max. length (*cm*) Range	V–L correlation r	m	C[h]
15	Petromyzontidae	17	60.02[i]	51.0–71.5	27.83	13–89	0.6056[b]	0.1115	41.43
89	Scyliorhinidae	12	134.49	109–186	63.76	30.2–304	0.5834[d]	0.1489	72.44
90	Carcharhinidae	56	168.83	114.7–244.6	143.98	24.2–1049	0.6981[a]	0.1738	71.16
91	Sphyrnidae	6	176.82	120–205.5	375.34	152.4–1059	0.8367[d]	0.2490	40.40
93	Squalidae	24	94.26	60–126	65.02	12.8–200	0.7477[a]	0.2329	36.65
99	Rajidae	23	90.46[j]	65.8–122.4	79.25	36.9–151	0.5055[c]	0.2595	29.08
192	Clupeidae[k]	139	46.12	37–61	18.64	3.5–76	0.6802[a]	0.1082	33.62
193	Engraulidae	74	43.67	38–75.5	11.73	3.3–60	0.4848[a]	0.1251	32.10
205A	Salmonidae[l]	19	62.78	54.5–69.5	87.67	14.1–155.1	0.3262	0.0353	53.61
205	Coregonidae[m]	32	59.46	53.2–66.6	46.66	16.7–152	0.5904[a]	0.0657	46.19
209	Osmeridae	11	62.11	54.5–68.5	20.45	9.8–40	0.6053[d]	0.1018	45.68
210	Argentinidae	9	48.28	40.5–66	20.51	11.3–55	0.6514[d]	0.1703	28.86
211	Bathylagidae	12	47.54	41–54	14.06	9.1–21.5	0.0562	0.0140	45.80
214	Salangidae	5	64.55	50.9–73.5	9.64	6–13.7	0.9539[b]	0.3608	28.50
220	Esocidae	5	57.16	46.4–65	100.85	38–182	0.9353[b]	0.2161	21.09
221	Gonostomatidae	23	40.82	31.5–63	9.70	3.1–61	0.3075	0.0650	35.21
222	Sternoptychidae	12	34.99	29.5–39	6.09	2.6–10.5	0.7146[b]	0.1527	26.55
223	Stomiatidae	18	60.38	39–164	16.44	5.3–43	0.7858[a]	0.4163	18.82
226	Melanostomiatidae	18	53.64	44–65	14.21	4.9–32.3	0.3306	0.0620	45.50
242	Synodontidae	14	54.30	44–63	33.83	9.8–64	0.6563[b]	0.1576	31.16
247	Paralepididae[n]	21	81.77	59.5–116	14.99	4.5–49.5	−0.1769	−0.0469	92.84
250	Myctophidae	16	37.23	33–41	9.07	2.6–19	−0.1916	−0.0260	39.42
260	Mormyridae	32	47.59	38.1–64	28.60	9.2–120	0.7430[a]	0.1648	27.38
261	Characidae	24	39.14	34–50	15.10	2.1–80	0.8915[a]	0.1170	28.49
266	Citharinidae	26	40.98	30–50	15.98	2.3–84	0.4182[d]	0.0487	35.81
271	Catostomidae	25	42.38	35.5–51	43.54	15–104	0.1530	0.0231	38.85
272	Cyprinidae[o]	272	40.12	28–58	18.09	2.8–122	0.3097[a]	0.1051	29.59
283	Bagridae	11	45.36	41.0–54.2	44.12	8–100	0.0052	0.0006	45.25
285	Ictaluridae[p]	32	37.31	31.3–52	13.46	4–132	0.8458[a]	0.1085	28.14
295	Mochokidae[q]	17	37.62	30.8–44	21.72	3.1–47	0.8133[a]	0.1059	27.15
319	Congridae	26	139.83	115–160.5	56.66	14.3–240	0.4267[d]	0.0645	107.76
335	Belonidae	9	76.86	53.5–91.5	49.91	4–152	0.9195[a]	0.1278	46.63
338	Exocoetidae	17	44.33	36.5–51.5	27.49	14.2–48.5	0.6764[b]	0.1857	23.96
340	Moridae	12	54.79	52.0–59.9	43.22	34–66	0.5183	0.1485	31.32
342	Gadidae	36	54.36	42–82	62.90	15–200	0.5167[b]	0.1202	33.04
354	Syngnathidae	113	55.09[r]	27.5–111.5	16.02	3.2–60	0.5392[a]	0.2074	30.99
362	Cyprinodontidae	27	30.63	24.6–37	5.29	2.1–15	0.6789[a]	0.1497	23.87

TABLE 1 (*Continued*)

Family No.[e]	Name	No. of species	Vertebrae Mean[f]	Vertebrae Range[g]	Max. length (*cm*) Mean[f]	Max. length (*cm*) Range	V–L correlation *r*	*m*	C[h]
367	Poeciliidae	34	30.54	28.0–34.3	5.64	2–13	0.0567	0.0073	30.15
392	Sphyraenidae	11	24.00	24–24	64.18	30–183	0	0	24.00
394	Atherinidae	26	43.21	31–52	13.51	5–46	0.7064[a]	0.1208	31.55
400	Centropomidae	16	24.18	24–25	52.76	18–320	0.7597[a]	0.0137	22.91
401	Serranidae[s]	75	24.33	24–26	33.59	3–229	−0.3522[b,s]	−0.0121	25.45
401A	Percichthyidae[s]	23	27.76	25–35.9	48.69	8–263	−0.2129	−0.0331	31.57
409	Centrarchidae	26	30.05	24.5[z]–33	20.82	3–63.5	0.8345[a]	0.0862	23.13
413	Percidae	65	39.45	33–48	10.33	4–137	0.5247[a]	0.0530	34.85
422	Carangidae	29	24.25	24–26	46.54	19.9–183	0.1902	0.0072	23.59
426	Bramidae	10	42.55	40–51.2	42.87	8.9–122	−0.0912	−0.0089	43.99
431	Lutjanidae	26	24.06	23.6–26	34.36	9–91.5	−0.4862[c]	−0.0139	26.82
435	Pomadasyidae	12	25.77	24–27	32.84	20–60	0.0471	0.0057	25.26
436	Sciaenidae	48	24.66	23–28	37.71	8.7–190	−0.1730	−0.0099	24.88
438	Sparidae	14	24.00	24–24	44.17	28–90	0	0	24.00
447	Kyphosidae[t]	4	26.87	26.5–27	44.28	24–64.5	0.9473[c]	0.0205	24.86
458	Cichlidae	76	29.86	25.7–38	16.45	6.6–75	−0.0294	−0.0039	30.20
460	Embiotocidae	21	27.12	31–38.1	34.63	14.9–58	−0.1167	−0.0565	40.32
462	Labridae	44	28.39	23–41	33.36	5–214	−0.0213	−0.0049	28.88
494	Blenniidae	50	34.63	29.8–41	6.68	2.2–34	0.0963	0.0103	33.96
501	Clinidae	19	41.67	32–57	11.67	3.5–61	0.5424[c]	0.1121	31.64
503	Stichaeidae[u]	20	65.97	43–82.5	22.62	7.5–76.5	0.3668	0.0941	49.19
504	Pholidae	12	86.44	61–105.7	21.44	9.5–46	0.4284	0.1576	53.32
506	Microdesmidae	10	54.40	41.5–63.2	10.47	6–25.7	0.7834[b]	0.2459	30.53
508	Zoarcidae	21	104.49	83.5–147	29.92	10.9–74.5	0.3123	0.0296	94.48
515	Ophidiidae[v]	16	58.02	40–87.9	20.77	8.9–46	0.5430[d]	0.1938	31.79
517	Ammodytidae	10	67.28	56–74.3	21.30	11.4–35	0.6613[d]	0.1709	39.88
524	Trichiuridae	13	112.14	58–191	82.08	20.4–183	0.5674[d]	0.3220	27.13
525	Scombridae[w]	40	39.29	31–60	82.85	18.8–300	0.5480[a]	0.1439	20.80
534	Stromateidae	21	31.01	25–46	23.31	8–50.8	0.2179	0.0782	24.25
535	Nomeidae	19	30.79	26–41	24.73	3.7–122	−0.4040	−0.0707	38.64
536	Anabantidae	17	27.08	25–30.5	10.51	3.5–61	0.2289	0.0199	25.85
541	Gobiidae	36	29.64	25–37.5	10.91	2.5–61	−0.1186	−0.0194	31.04
544	Scorpaenidae	119	26.16	23–30.5	26.60	4.9–92	0.1742	0.0514	22.10
545	Triglidae	11	33.83	27–37	38.23	15.2–61	−0.2309	−0.0472	40.17
550	Hexagrammidae	13	50.96	37.5–61	45.47	25.5–152	0.7046[b]	0.2253	21.56
555	Icelidae	11	34.91	32.9–38.3	15.60	8.5–27	−0.0571	−0.0069	35.58
556	Cottidae	90	34.62	24–55	16.01	5.1–63.5	0.3159[b]	0.0656	28.86
557	Cottocomephoridae	11	32.50	29–36	14	5.5–22.5	0.6802[c]	0.0938	25.43

TABLE 1 (*Concluded*)

Family No.[c]	Name	No. of species	Vertebrae Mean[f]	Vertebrae Range[g]	Max. length (*cm*) Mean[f]	Max. length (*cm*) Range	V–L correlation *r*	*m*	C[a]
562	Agonidae	15	39.59	35.5–46	17.47	9–31	0.0532	0.0144	37.99
563	Cyclopteridae[x]	10	27.20	23.5–29	13.02	3.9–61	0.8047[b]	0.0704	22.70
563A	Liparidae[x]	17	51.38	35.2–70	17.41	5.9–46	0.3514	0.1533	33.16
568	Bothidae[y]	90	38.25	33–55	19.71	6.6–152	0.0860	0.0169	36.36
569	Pleuronectidae	46	41.68	29–64.2	48.43	13–266.7	0.3859[b]	0.1304	25.12
570	Soleidae	30	33.55	24–49.7	17.47	7–51	0.1391	0.0574	28.47
581	Balistidae	14	19.84	17–23	32.94	6–101	−0.1916	−0.0278	21.87
583	Tetraodontidae	22	18.70	17–22	31.66	11.5–76	0.2557	0.0373	16.44
586	Gobiesocidae	32	31.19	26–43	4.70	2.1–30	−0.1661	−0.0376	33.06
587	Batrachoididae	11	32.89	26.5–47.6	14.75	5.4–48.5	0.7759[b]	0.2396	17.26

[a]$P < 0.001$; [b]P 0.001–0.01; [c]P 0.01–0.02; [d]P 0.02–0.05; Others $P > 0.05$; [c]Family numbers are those of Berg (1940) except as noted below; [f]Geometric means of species means; [g]Range of species means; [h]Intercept at L = 1; [i]Myotome counts; [j]Excludes vertebrae posterior to first dorsal fin insertion; [k]Excludes Dussumieriini; [l]Includes only subfamily Salmonini of Berg; [m]Subfamily Coregonini of Berg; [n]Includes only subfamily Paralepidini of Berg's family Sudidae; [o]Within Cyprinidae, the groups Cyprinina, Leuciscina, and Abraminida each also shows a highly significant *r*; [p]Amiuridae of Berg; [q]Synodontidae of Berg; [r]Total of bony dermal rings on trunk and tail; [s]If Percichthyidae is included in Serranidae (following Berg (1940) and Greenwood et al. (1966)), *r* is not significant; [t]Girellidae of Berg; [u]Includes Lumpenidae of Berg; [v]Includes Brotulidae of Berg; [w]Includes Cybiidae and Thunnidae of Berg; [z]If Liparidae is lumped with Cyclopteridae (following Berg), *r* is not significant; [y]Excludes Citharidae and Scophthalmidae; [z]Includes suspiciously low counts for *Elassoma* from Jordan and Evermann 1886–1900.

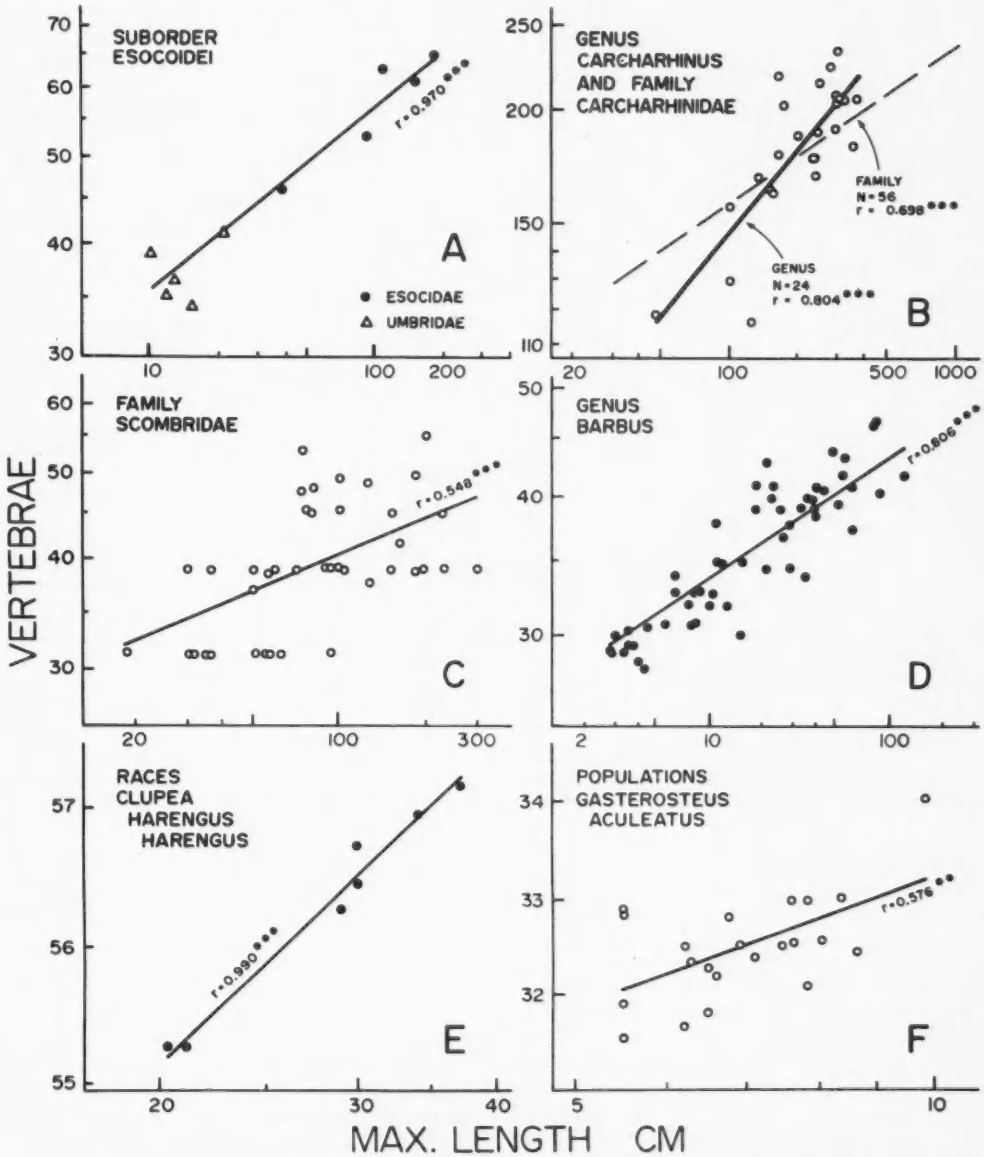

FIG. 1. Relation of mean vertebral number to maximum recorded length in representative taxa. Each point represents a species (A–D), race (E), or lake population (F). *** $P < .001$; ** P .01 – .001.

associated with considerable difference in the relative body depths (and widths) between species. Often a species having fewer vertebrae than would be predicted from its length is found to have a proportionally deeper body than its relatives. The more the variation in relative body depth with a family, the less close is the correlation between V and L.

Taxonomic inadequacies probably affect the reliability of some of the calculated values. For example, several eel families (order Anguilliformes) other than Congridae are suspected to

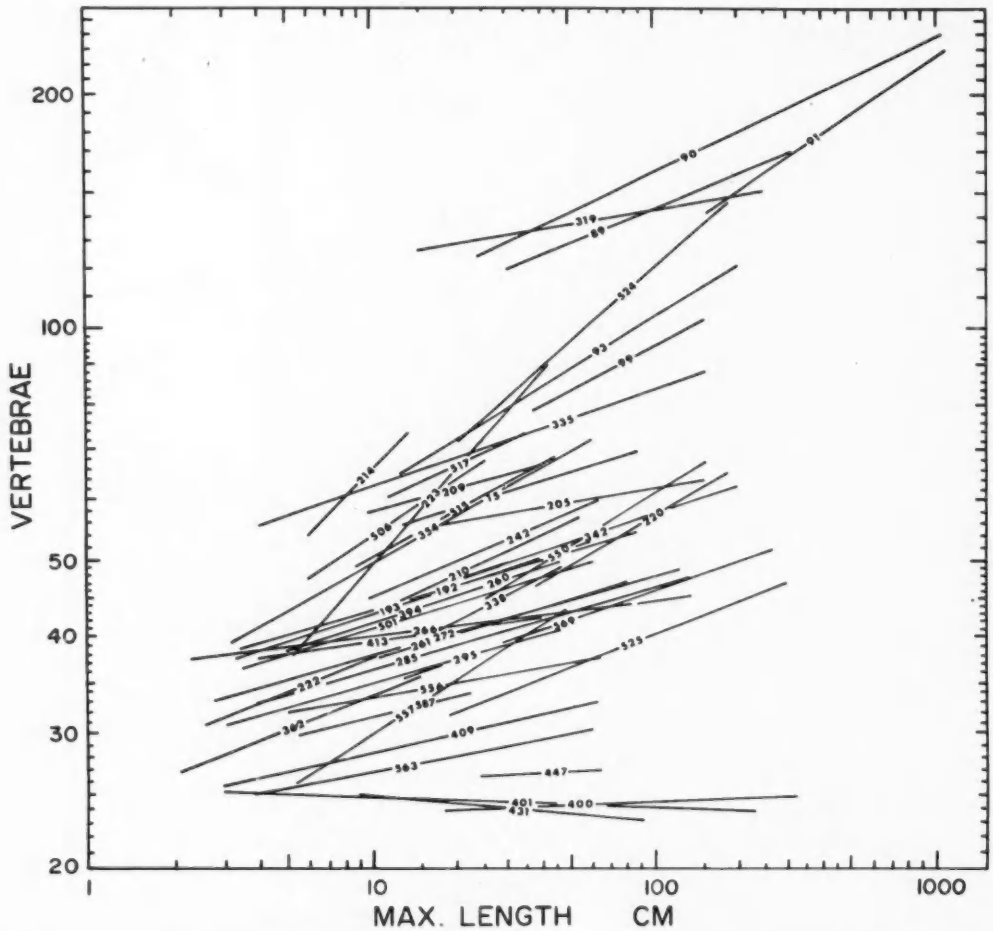

FIG. 2. Regressions of vertebral number on maximum length for all families with statistically significant correlations. Family code numbers (corresponding to Table 1) are located at the geometric means of vertebral number and of maximum length; ends of each regression line indicate the range of maximum lengths.

include unresolved synonymies between large and small "species." The significantly negative slope in the family Serranidae as here recognized disappears if Serranidae and Percichthyidae are lumped (as was done both by Berg (1940) and Greenwood et al. (1966)). Treatment of all species in a family without regard to latitude may obscure pleomeristic trends within latitudinal zones, as is shown later for the wrasses (family Labridae).

Above the family level — Figure 2 demonstrates that while there is an overwhelming tendency for a positive correlation to occur between V and L within families, no such clear relationship exists among the whole array of fishes. Although family means show a very rough trend towards positive correlation between V and L, there are wide departures. For example, salmonids are 7 times as long as salangids but have about equal vertebral numbers; congrid eels are about the same mean length as centropomids but have 6 times as many vertebrae. In general, the within-family correlation between V and L can be expanded between families only to the extent that the fishes included have similar body form and structure. The order

Esociformes (Fig. 1A) demonstrates that fishes in two families which have rather similar body outlines (small mudminnows, Umbridae, and large pikes, Esocidae) may follow the same V–L trend; other pairs or groups of families which are structurally and phyletically close may likewise have similar V–L trends (e.g. herrings, Clupeidae, and anchovies, Engraulidae; North American catfish, Ictaluridae and upside-down catfish, Mochokidae). But there is no such correlation between families if their body shapes differ markedly despite taxonomic affinity; as in the within-family comparisons, there is a general tendency for the families of more robust fishes to have the lower vertebral counts. For example, lumpfish, Cyclopteridae, which are rotund, have markedly fewer vertebrae than the similar-sized and phyletically related snailfish, Liparidae, which are slimmer. A striking disparity separates the egg-laying killifish family, Cyprinodontidae, which has strong pleomerism, and the related family of livebearers, Poeciliidae, which has none. These two families share the same means and ranges, both in size and in vertebral number, and have roughly similar shapes, but they differ in reproductive methods and associated structural modifications; in Poeciliidae the females almost always reach a much larger size than the males. Another viviparous family, the surfperches, Embiotocidae, also show no significant pleomerism. But viviparity is not invariably linked with lack of pleomerism, as is demonstrated (Fig. 1B) by the viviparous requiem sharks *Carcharhinus*.

The steepness of slopes of the V–L lines can be seen in Fig. 2 to tend to increase in families with high mean vertebral counts. Correlation between the exponents m and the mean vertebral counts is significant (P .01–.02) for the 47 families shown in Fig. 2, and highly significant ($P <$ .001) for the 85 families in Table 1. Most families with low counts (including several in the order Perciformes) display pleomerism only weakly; in the extreme, some families have an invariable vertebral number regardless of body length (e.g. 27 in Platycephalidae; 24 in Sparidae, Sphyraenidae and Mullidae; 21 in Callionymidae). The two families with statistically significant negative correlations, Serranidae and Lutjanidae, have small overall ranges of variation in vertebrae. Taxonomic limits of Serranidae are debatable. The weak negative slopes in these two families may be doubted to have biological significance. The steeper slopes, all positive, occur mostly (but not exclusively) in families with high overall vertebral counts. The median value of m for families shown in Fig. 2 is 0.12 which represents about a 10% increase in vertebral number for each doubling in length. The steepest slope (m 0.42 for Stomiatidae) is a 32% vertebral increase for each doubling in length. Steepness of slopes in different families shows no significant correlation with their mean body lengths, independent of vertebral means.

Correlations between vertebral (or myotome) number and maximum body length, which have been demonstrated above to occur in the Class Agnatha, Chondrichthyes, and Osteichthyes, probably also occur in others. In 27 species of Cephalochorda, family Branchiostomatidae, there is a tendency for myotome number to increase with body length (m 0.067), although scatter is wide and the correlation is not statistically significant. In 149 species of limbless Amphibia in the order Caecilia there is striking pleomerism, provided comparisons are confined to species with roughly the same relative body widths. In Reptilia, an initial survey of the sea snake family Hydrophiidae suggests a pattern similar to that in the caecilians, although relative body width, which again has a critical influence, is poorly documented. I have not yet surveyed other vertebrate or invertebrate groups.

Within genera — In keeping with the observation that the V–L correlation is highest among fishes of closely similar form, the most striking correlations are found in species belonging to the same genus. In the cyprinid genus *Barbus* (which has more species than any other genus of fishes) the level of significance of the V–L correlation in 54 species is very high indeed (Fig. 1D). Often the scatter of points within a genus is less than in the whole family, as in the shark genus *Carcharhinus* (Fig. 1B). Significant correlations were found in the following other genera (as well as in their families): *Raja* (Rajidae), *Alosa* and *Clupea* (Clupeidae), *Esox* (Esocidae, Fig. 1A), *Gnathonemus* (Mormyridae), *Notropis* and *Leuciscus* (Cyprinidae), *Ictalurus* and *Noturus* (Ictaluridae), *Synodontus* (Mochokidae), *Conger* (Congridae), *Cypselurus* (Exocoetidae), *Syngnathus* (Syngnathidae), *Percina* (Percidae) (also found by Page, 1974), *Cottus* (Cottidae), *Citharichthys* (Bothidae) and *Pleuronichthys* (Pleuronectidae).

Occasionally a genus may display significant pleomerism among its species, while the family as a whole does not. The sucker family Catostomidae includes some relatively robust and deep-bodied forms (*Carpiodes*, *Ictiobus*) whose vertebral number is lower than that of more slender relatives having comparable maximum lengths. As a result, the family as a whole does not show a statistically significant V–L correlation; yet

among species within the contained genus *Catostomus*, which are relatively uniform in body shape, V and L are highly significantly correlated. Similarly the genus *Haplochromis* has a significant correlation, while its family, Cichlidae, does not.

Within species — The tendency for pleomerism is evident within as well as between species, although data are scarcer. A few examples will illustrate the widespread but rather diffuse evidence.

Seven separate spawning races of European herring *Clupea harengus harengus* (Fig. 1E) display a significant correlation ($P < 0.001$) between mean vertebral count and maximum recorded length (data from Andriyashev 1954, Hempel and Blaxter 1967, Schnakenbeck 1931, and Svetovidov 1952). The two points in the lower left of Fig. 1E represent Baltic races that spawn in brackish water, but even if these are omitted, the remaining five races that spawn in higher salinities still show a significant correlation ($P < 0.02$). Data are available on a larger number of races for mean body length of spawners (rather than maximum length); here, in 14 races, there is again a significant correlation ($P < 0.02$) with vertebral means. It may be of taxonomic significance that V–L correlations break down completely if races of the Atlantic herring and of the Pacific herring (sometimes considered as a separate species *Clupea pallasi*) are lumped together in the analysis; the 12 races of Pacific herring show a weakly positive correlation among themselves, but all have much lower vertebral counts than any of the Atlantic races. Despite this discrepancy between Atlantic and Pacific groups of races, the genus *Clupea* as a whole shows significant pleomerism between species, as does the family Clupeidae.

The sand lances *Ammodytes* of the northwest Atlantic also display pleomerism. Comparing 10 areas on the eastern North American seaboard (from Newfoundland to Massachusetts), and following Scott's (1972) treatment of these as members of a single species, there is a significant correlation between mean vertebral count and maximum length ($P < 0.01$). In many localities within the region there are two groups, "inshore" and "offshore" types (treated as two species by Richards et al. 1963). Where the two occur together, the "offshore" types, which are more slender, usually have the higher vertebral count (seven of eight comparisons), and larger average size (seven of nine comparisons).

Large samples of the redfish *Sebastes marinus* have been examined in the region extending from Labrador to Gulf of Maine (Templemann and Pitt 1961). Comparing redfish either from 31 areas or from 9 grouped areas, the mean vertebral counts are significantly positively correlated with maximum recorded body length ($P < 0.05$).

In Japan, loach *Misgurnus anguillicaudatus* from six localities also show a significant correlation ($P < 0.05$) between vertebral count and maximum body length (Kubota 1961; Kubota and Ono 1965).

In each of the preceding four examples vertebral numbers and also body sizes tend to increase from south to north, so that vertebral count and size cannot be shown to be correlated independent of latitude. Data are available, however, from 22 populations of threespine sticklebacks, *Gasterosteus* (*aculeatus?*), all inhabiting small lakes on Graham Island of the Queen Charlotte Islands (G. E. E. Moodie personal communication). Since all the lakes although isolated are within a few miles of each other, no latitudinal (nor significant altitudinal) effect is involved. Maximum body lengths vary considerably between the different populations, and again can be seen, in Fig. 1F, to be positively correlated with mean vertebral numbers ($P < 0.01$).

In many instances a "dwarfed" form (often landlocked or confined to headwater streams) has lower vertebral counts than the normal-sized form of the same species (often anadromous, or lacustrine). Thus, the small nonmigratory "kokanee" form of *Oncorhynchus nerka* typically has fewer vertebrae than its progenitor, the large sea-going sockeye salmon. Kokanee have probably developed from sockeye independently in many different places. McCart (1971) showed that in spawning samples from each of six tributaries to Babine Lake, B.C., the sympatric sockeye and kokanee always differed significantly in vertebral number; the lower counts of the kokanee persisted when eggs from the two forms in one stream were incubated under the same hatchery conditions.

Comparing anadromous populations of chum salmon, *Oncorhynchus keta*, Kubo (1956) showed that the adults were larger in one spawning population (in Shiriuchi River) than in other rivers of Hokkaido, and that these also had a higher mean vertebral count. Again, under similar hatchery rearing conditions the offspring from the larger Shiriuchi fish produced more vertebrae than did the offspring from one other river system.

In rainbow trout, *Salmo gairdneri*, Northcote et al. (1970) compared a population of small trout that inhabited a mountain stream above an impassable waterfall, with their larger relatives inhabiting the lower section of the stream which enters Kootenay Lake, B.C. The headwater form with smaller body length had significantly fewer

vertebrae (as well as more scales, more parr marks, and different genotype frequencies for liver LDH isozymes). Earlier experiments (Mottley 1937) had established that the difference (between vertebral counts of comparable headwater forms in the region and of the large Kootenay Lake form of trout) persisted even in offspring reared in a hatchery.

McDowall (1972) showed striking differences in vertebral count between diadromous populations of *Galaxias maculatus* and their landlocked derivatives in Australia, New Zealand, and South America. The larger anadromous forms averaged in some cases as many as 11 vertebrae more than their smaller lacustrine relatives.

Many other examples of intraspecific pleomerism can be found. Wherever appropriate data have been available for several different races of one species, the correlation between size and vertebral count if it is statistically significant has been positive.

Between the sexes — A few cases are known in which difference in body length between the sexes is accompanied by a difference in total vertebral count. Blennies of the genus *Ecsenius* offer a striking example (Springer 1971). In *Ecsenius bicoler*, *E. namiyei*, and *E. frontalis frontalis* the maximum recorded lengths of the males exceed those of the females (by 30, 23, and 16%, respectively), and in all three species the males also have significantly more vertebrae than the females (by 1.2, 1.7, and 0.5%).

The opposite sexual difference occurs in the small shark *Etmopterus spinax* (= *Spinax niger*), in which adult females have significantly more total vertebral segments than have adult males by 0.7% (Punnett 1904). Maximum lengths of the sexes are not available, but Wheeler (1969) states that females mature at 36 cm and males at 33 cm; again, the larger sex has the higher vertebral number.

The case of the capelin *Mallotus villosus* is less clear. In capelin of British Columbia (which were once referred to as a distinct species (*M. catervarius*), males are longer and heavier than females, and males also have significantly higher vertebral counts (by 0.6%). In capelin from the Murmansk region vertebral counts of males are also well above those of females. But in Newfoundland, females have slightly but significantly higher vertebral means than males (by 0.1%); here, although the largest individuals recorded have been females, the males grow faster, and as in B.C. the average length of males in most samples of spawners exceeds that of females (Hart 1973; Jangaard 1974). The apparent contradiction may arise because males have substantially deeper bodies than females (at least in the posterior region), which may tend to offset the effect of greater average body length, and sex difference in body depth is perhaps more pronounced in the western Atlantic than in the Pacific capelin.

Despite the foregoing examples, sexual dimorphism in total vertebral count has not been found in the great majority of species in which it has been sought. But the proportion of abdominal to caudal vertebrae is sometimes higher in females, presumably conferring a greater egg-carrying capacity. In *Mallotus villosus* from British Columbia, even though the males (which are longer) have higher total vertebral counts, the females have more abdominal vertebrae (Hart and McHugh 1944).

Vertebral Number and Latitude

The tendency for fishes in polar or cold waters to have more vertebrae than related forms in tropical or warm waters has long been known, and is sometimes dignified as "Jordan's rule" (see Terminology). Another tendency has been demonstrated, for the proportion of fish species with large maximum size to increase from the equator towards the poles, within some families as well as between whole faunas (Lindsey 1966). Since it has now been established that large species tend to have more vertebrae (pleomerism), the question arises whether latitudinal clines in vertebrae (Jordan's rule) may be purely a reflection of latitudinal clines in body size.

One way to analyze separately the relationships of vertebral count, maximum body length, and latitude is to examine the correlations of vertebrae and length independently for fish occurring in different latitudinal zones. In Fig. 3, for each of two families, the V–L line and the geometric mean is shown for all species that occur in the Arctic zone (top line), all that occur in the Temperate zone (middle line), and all in the Tropical zone (bottom line). (In these calculations, a wide-ranging species may be included in two or even all three zones. A different calculation, which assigned each species to a single zone on the basis of its midrange latitude, gave somewhat different V–L slopes but led to the same interpretations.)

In the herring family Clupeidae (Fig. 3, top), for each zone the slope of the line indicates that V and L are positively correlated. The mean counts and mean lengths for all species in each of the three zones also show a positive correlation. Hence the differences in mean maximum lengths between latitudes reinforce the latitudinal cline in vertebral number. But, the Arctic line is

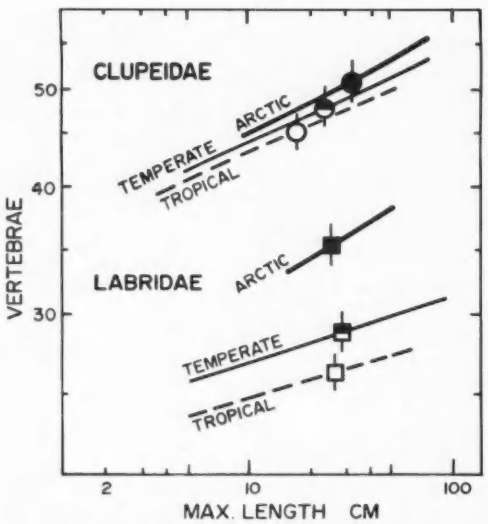

FIG. 3. Regressions of vertebral number on maximum length in families Clupeidae and Labridae, each separated into latitudinal zones: Arctic 50°N–64°N), Temperate (25°N–49°N), and Tropical (24°N–24°S). Symbols show geometric means.

displaced vertically compared to the Tropical line, indicating that for fishes of the same length, northern forms have more vertebrae on the average. Another method of analysis (not shown) was to compute for each 5° latitudinal zone the regression of vertebral count on length for all species in that zone. From these regressions, an adjustment was applied to the mean vertebral count for that zone so as to remove the effect of length. A strong latitudinal cline persisted in the adjusted vertebral numbers.

These and other methods of analysis all show that in several different fish families a distinct latitudinal cline in vertebral number remains after the effect of maximum length is removed. Families showing such clear clines include Catostomidae, Cyprinidae, Gadidae, Scorpaenidae, Cottidae, and Pleuronectidae.

A striking demonstration of a latitudinal cline in vertebrae which can not be attributable to a cline in maximum body lengths is provided by the wrasse family Labridae (in which the phenomenon of vertebral clines was first noticed by Günther in 1862). Wrasses are unusual in that the average maximum size of species does not increase from the tropics to the arctic (Fig. 3, bottom). Despite the lack of a latitudinal cline in maximum length, there is, nevertheless, a marked increase in vertebral numbers from south to north. The sharp difference in vertebrae at different latitudes is so great as to overshadow the rather weak (nonsignificant) positive correlations between vertebrae and length within each zone (Fig. 3), so that when all species are analyzed jointly without regard to latitude the V–L correlation is not significant (Table 1).

In summary, Jordan's rule cannot be attributed solely to latitudinal clines in maximum body sizes. The possibility is raised in the Discussion that pleomerism may, nevertheless, be implicated with respect to some other body size whose value is affected by the shape of the survivorship curve.

Discussion

Previous Literature

Some aspects of pleomerism have been described previously, even though the widespread nature of the phenomenon seems not to have been recognized. Several authors noticed that very small species have fewer parts. Jordan (1905: 212) wrote "In some groups of fresh-water fishes there is a reduction in number of vertebrae, not associated with any degree of specialization of the individual bone, but correlated with simple reduction in size of body." Myers (1958) noticed that, in a number of families, those species with minute adult size typically showed reduced numbers of scales and of fin rays, although he did not mention numbers of vertebrae. Liem (1963) suggested that reduction of vertebral number in two genera of anabantid fishes may be correlated with their small body size. Briggs (1969) described a tiny clingfish as one of the smallest vertebrates ever found and noted that its vertebral count was 25 in contrast to 34–37 in a related genus comprised of larger species.

In his analysis of vertebral variation, Kyle (1926: 188) apparently considered, simultaneously, very differently shaped sorts of fishes, and consequently wrote, "It might be thought that the number of vertebrae depended on the ultimate size of the fish, but this is far from being the case; the largest fish are among those with the smallest number of vertebrae." Kyle noted that the number of vertebrae varies very closely according to the length of fish "during the early postlarval stage," but he went on to state that adult size has nothing to do with larval length.

Rass (1948) also observed, in *Liparis* and in certain other marine fishes, a correlation between vertebral number, egg size, and length of the larvae when they differentiate, all of which values increase from the tropics towards the pole. He noted, however, that in species of *Liparis*, body size increases between the south boreal and arctic

Boreal zones, but then decreases from the polar front into the Arctic; he concluded (translated from Russian) that "the number of vertebrae increases from the Tropics toward the pole (Jordan's rule) independently of the size of individuals." Rass's statement is therefore in agreement with the present conclusion that Jordan's rule may occur independent of pleomerism, but Rass did not observe that the converse is also true. Garside (1970) wrote with respect to latitudinal clines in meristic series, "The phenomenon, whatever might be its explanation, is not related to size trends in fishes since both very large and very small species of acanthopterygians have low vertebral number." Berg (1926) gave several examples of fishes, showing a progressive increase, from warm to cool latitudes, both in vertebral number and in length of body; similar observations by other authors have already been cited. None of these publications has referred to the pleomeristic relationship independent of latitude.

The clearest interpretation of vertebral variation along the present lines was given by Bailey and Gosline (1955) who recorded vertebral counts of species in the family Percidae. They concluded, "In the American percids a highly significant relationship seems to exist between vertebral count and adult size. . . . Decreased size is apparently, and probably causally, correlated with reduction in vertebral number." They did not attempt to quantify "adult size" of the species examined, and hence made no calculation of the degree of correlation, but they quite clearly perceived within the family Percidae the phenomenon referred to here as pleomerism. Recently Page (1974) has analyzed 32 species in the genus *Percina* within this family and also finds that low vertebral count is associated with small maximum body size. Lindsey and Ali (1971) referred in a preliminary way to some of the data in the present contribution.

The Pleomeristic Rule and its Explanation

This analysis has demonstrated that pleomerism is a widespread phenomenon, found in the majority of families of fishes as well as in some taxa above and below the family level. Vertebral number (V) is positively correlated with maximum body length (L) in 90 of the 118 fish families examined with $n > 4$. The power function $V = CL^m$ has very different values of C and m in different families, and yet it describes the relationship of V to L in each of a variety of groups that differ radically in shape and habit. Pleomerism occurs in swift mackerels, slow seahorses, large sharks, tiny killifish, slender eels, deep-bodied sunfish, rotund lumpfish, flatfish which swim on their sides, rays which swim with their wings, and mormyrids which swim with their bodies straight. Moreover, amphibians (caecilians), and reptiles (some groups of snakes) display pleomerism, provided species of similar robustness are compared. Pleomerism may be even more widespread; invertebrates have not yet been examined.

Such a pervasive phenomenon may for convenience be designated as the Pleomeristic Rule: namely, that among related taxa the number of segments is correlated with the characteristic body size. "Rules" such as this, like Bergmann's Rule and Allen's Rule, lack the purported precision of many of the "Laws" of physics; they are merely "descriptive generalizations of low-order probability that describe some common regularities without explaining anything" (Gould 1970). But despite its rather low predictive power the Pleomeristic Rule seems to apply so widely as to suggest a common underlying cause, which should eventually be uncovered.

The term pleomerism is not tied to any particular mathematical expression. Choice of the power function has been purely empirical. Arguments for using it, rather than more complex mathematical expressions, are similar to those clearly presented by Gould (1966) to justify the power function as an expression of allometric growth. See Gould also concerning the dangers of attributing spurious biological meaning to the constant C, because of its interdependence with the exponent m and with the units of measurement, and because the interception with $L = 1$ is an extrapolation beyond the actual size range.

Some features of the data have been pointed out, and many more may be found by inspection. The rate at which vertebral number increases with body length tends to be great in families with high overall vertebral counts, and is least, often zero, in families with low overall counts. The trend for steeper V–L slopes to accompany higher overall vertebral counts is further extended if caecilians and sea snakes are considered. Slopes tend to be greater in fish groups usually considered to be more primitive, and in those having less complete ossification of the vertebral column. Large overall body size does not always go with steep V–L slopes (e.g. Centropomidae), and families with small body size may have steep slopes (e.g. Salangidae). Slim species tend to have higher counts than robust species, a general trend noticed by many other workers and present both within and between families. Discovery of two families with statistically significantly negative V–L correlations is perhaps without biological

significance, in view of the large number of families examined which, like these two, had few vertebrae and little overall variation in vertebral number.

Pleomerism is absent in two viviparous families (Poeciliidae, Embiotocidae) but present in one viviparous genus of sharks (*Carcharhinus*). These and other data suggest that egg size may be implicated in pleomerism, since large fish within a family often tend to lay larger eggs. But no causal connection during embryogenesis has been shown between yolk size and vertebral count in *Oryzias latipes* (Lindsey and Ali 1971). Moreover, vertebral differences between sexes of different size can scarcely arise from egg size. The subject needs further exploration.

Many possible causes of pleomerism could be suggested. If pleomerism arises from some functional advantage, the structures directly involved may be the vertebrae, or the myomeres, or the nerves. Perhaps the proportions of the individual vertebrae vary with body size in response to requirements of mechanical strength. Hydrodynamic analysis of fish propulsion (Webb 1975) suggests that fish of the same shape but different sizes may have different swimming patterns. If flexibility is involved, perhaps long animals swim with sharper curves, or in large fish each intervertebral joint may confer less flexibility and hence more are required. Perhaps the folding of the myomeres is involved; perhaps there is an optimum length of muscle fiber. Perhaps there is no functional advantage in the correlation of adult size and segment number; maybe instead the larval size is critical, or maybe pleomerism arises indirectly from a relation between adult body size and egg size. Some of these hypotheses are readily testable; some are not. They will not be explored further in this discussion, in part because I am as yet unable to offer any hypothesis that seems satisfactory.

Some Consequences of Pleomerism

Even without understanding its cause, the empirical observation that within a group of species vertebral number is significantly correlated with adult size may sometimes be useful taxonomically. Calculation of the pleomeristic formula for those species already known in a genus or family allows one to predict the probable adult size of a newly discovered species from its vertebral count, even when only young individuals are available. Suspiciously high vertebral number in a few small rare specimens may suggest either that there are as yet undiscovered much larger adults, or that one "species" described on the basis of small specimens in fact represents the young of a known larger species. Several such instances arose while assembling data in this study; displacement of a point alerted me to look for, and sometimes discover by further literature search, a synonymy between small and large named species. Vertebral count of a fossil specimen could suggest the maximum size the form probably attained, provided the pleomeristic constants were known for related modern forms. There may, however, have been evolutionary trends towards reduction in the number of parts in fishes.

Disparity between the V–L regression lines in two groups of taxa provides additional input for taxonomic judgment. Examples have been given concerning Atlantic and Pacific groupings of *Clupea harengus* and of *Mallotus villosus*. At a higher taxonomic level, wide departure from pleomerism within a family may suggest a reevaluation of the family limits (e.g. Paralepididae). Thus, vertebral count considered in conjunction with maximum size of the specimens available is more reliable for making taxonomic equations than is vertebral count alone. The technique is likely to be useful in large and confusing genera such as *Barbus* (Fig. 1D).

Other more exciting consequences of pleomerism may arise if, as suggested by the data, there is some functional correlation between body size and optimum number of segments. The fact that each fish is, so to speak, stuck with a single vertebral number that has to serve it at all sizes is in contrast to the changes in body proportion that commonly occur in ontogeny and that may compensate for changes in surface-to-volume and other size-dependent ratios. Bertalanffy (1964) wrote, "It is a truism in engineering that any machine requires changes in proportion to remain functional if it is built in different size, for example if a small-scale model is increased to the desired working size." Widespread occurrence of pleomerism suggests that the fish machine may also require changes in number of segments when it is built in different sizes. Such changes can be and apparently have been selected for between species with different adult sizes, but cannot occur between different ontogenetic stages of one individual. Vertebral number in fishes is fixed at or shortly after hatching and remains unchanged thereafter (except possibly in the snipe eels, Nemichthyidae). Hence, since each individual can have only one vertebral count at all stages, there will be a body size at which its vertebral count is functionally optimal; below this size the fish will have too many vertebrae for best performance, and above this size it will have too few.

One consequence might be that the mean

vertebral count of the survivors of a year-class of fish will decrease during the period from hatching until the mean count functionally matches the body size; after this size, the mean count of survivors should steadily rise, since selection will now be favoring progressively higher counts to match increasing size. The hypothesis could be tested by observation on wild or experimental fish.

Meristic variation within single fish populations is often striking and can have both phenotypic and genotypic components (Ali and Lindsey 1974). Fowler (1970) discusses the question of why selection tolerates so much more meristic variability in fish than in most animals, rather than favoring the single best-adapted number of parts. Possibly the answer is related to the fact that no one vertebral count can be best for all sizes; unpredictable shifts in the size on which selection operates most stringently might be met best by retaining a wide spectrum of meristic counts in each generation.

Although the size used in this analysis was the maximum length each species is known to attain, the more relevant size would probably be that at which selection with respect to vertebral count is most significant. Hence, differences in survivorship curves, even between populations reaching the same maximum size, might be expected to be reflected in differences in mean vertebral numbers, since selection will have matched mean vertebral count of each population to a slightly different body size. The outcome will be complicated by variation in the selective significance of vertebral count at different life history stages; for example, locomotory ability, which may be linked to segment number, is probably selectively neutral until the larva is free-swimming, and may also vary in importance between different later stages.

The latitudinal trends in vertebral count, which have been shown to persist even when the effect of maximum body length is removed, may reflect latitudinal differences in survivorship curves. Perhaps Jordan's rule holds, even independent of maximum lengths, because the age and size structure of cold-water populations represent a larger average body size to which vertebral count is matched.

The foregoing suggestions regarding selection are highly conjectural and involve many untested assumptions. Yet, perhaps, from experiment and from theory, it will be possible to calculate the optimal body length of a species corresponding to a given vertebral number. If so, then one might eventually assemble a model interpreting vertebral variation within a particular population in terms of varying selective pressures on different parts of the life history. The concept could be extended also to other meristic counts, and indeed to any other character which conveys different selective advantage at different body sizes but which, while varying between different members of a population, cannot vary during the life of a single individual.

The ubiquity of the phenomenon of pleomerism is striking; vertebral number seems to be tuned to body length not only between related species, but even between geographically adjacent populations and sometimes between the sexes. The inclusion of body depth or width as well as length in the analyses promises to account for even more of the variation in vertebral number. Further analysis and experiments may produce a functional explanation of pleomerism useful in understanding the mechanics of locomotion.

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Genetic Variation in the Pacific Oyster, *Crassostrea gigas*[1]

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BUROKER, N. E., W. K. HERSHBERGER, AND K. K. CHEW. 1975. Genetic variation in the Pacific oyster, *Crassostrea gigas*. J. Fish. Res. Board Can. 32: 2471–2477.

Genetic variation, exhibited by electrophoretic analysis of tissue proteins and enzymes, of the Pacific oyster (*Crassostrèa gigas*) was analyzed. Eleven systems were examined, reflecting 15 loci of which 8 were polymorphic and 7 were monomorphic. The number of alleles per locus varied from 1 at four loci (*Aat*-1, *Mdh*-1, *Me*, and *To*-1) without allelic variation, to 6 at the *Pgm* locus. The average number of alleles per locus was 2.7, while the effective number of alleles was calculated to be 1.3. An individual was found to be heterozygous, on the average, at 21% of the loci. Allelic variation was found at 11 out of 15, or 73% of the loci. At 53% of the loci the frequency of the most common allele was no greater than 0.95.

BUROKER, N. E., W. K. HERSHBERGER, AND K. K. CHEW. 1975. Genetic variation in the Pacific oyster, *Crassostrea gigas*. J. Fish. Res. Board Can. 32: 2471–2477.

Nous avons étudié par électrophorèse la variation génétique des protéines et des enzymes tissulaires de l'huître du Pacifique, *Crassostrea gigas*. Onze systèmes ont été examinés, montrant 15 loci, dont 8 sont polymorphes et 7 monomorphes. Le nombre d'allèles par locus varie de 1 à quatre loci (*Aat*-1, *Mdh*-1, *Me*-1 et *To*-1) sans variation allélique à 6 au locus *Pgm*. Le nombre moyen d'allèles par locus est de 2.7, alors que le nombre effectif d'allèles a été calculé à 1.3. On constate qu'un individu est hétérozygote, en moyenne, à 21% des loci. On observe une variation allélique à 11 sur 15, ou 73% des loci. A 53% des loci, la fréquence de l'allèle le plus commun ne dépasse pas 0.95.

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A genetic study was recently initiated in an attempt to develop a strain of oysters resistant to a disease apparently caused by *Vibrio* species (Lipovsky and Chew 1972; Grischkowsky 1973), as part of a Sea Grant program to investigate methods of reducing, and possibly eliminating, this mortality in the Pacific oyster, *Crassostrea gigas*. The initial part of this program has involved determining the extent of genetic variation available in various populations of the Pacific oyster, and attempting to characterize polymorphic gene loci associated with increased resistance. Although some genetic work has been done with this oyster species (Ahmed 1973; Lannan 1971, 1972; Menzel 1968), no single gene differences have been reported.

This report describes genetic variation in the Pacific oyster investigated by electrophoretic separation of proteins on starch gels, and presents an analysis of the variation found in a high mortality oyster population, based on the Hardy-Weinberg equilibrium hypothesis. The electrophoresis procedure allows an analysis of single gene differences between individuals, and gives an indication of the genetic diversity within and between populations. Evidence is presented for a high degree of polymorphism in 15 clearly defined loci. Possible explanations for this variation and for its application to oyster culture are discussed.

Materials and Methods

Specimens of *C. gigas* used in the electrophoretic analysis were collected from Mud Bay in southern Puget Sound near Olympia, Washington. These oysters were killed and dissected for the specific tissues required in the analysis (Table 1). The tissue was immediately wrapped in aluminum foil, placed in a whirl bag, and stored at −20 C. Just prior to electrophoresis, tissue samples were homogenized in an equal volume of 2% phenoxyethanol (Nakanishi et al. 1969). No further treatment of the samples, such as centrifugation or freeze-thawing, was found to be necessary for good electrophoretic separation of the proteins.

Horizontal starch-gel eletcrophoresis was performed by the methods of Kristjansson (1963). Three different buffer systems were used in this study:

A. Electrode buffer — 300 mM boric acid and 60 mM LiOH, pH 8.1 (Ridgeway et al. 1970)

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TABLE 1. Enzymes assayed, procedures used, and numbers of loci detected in a study of *Crassostrea gigas*.

Enzyme system	Abbrev.	Tissue[a]	Buffer System	Reference[b]	Number of loci	
					Polymorphic	Monomorphic
Aspartate aminotransferase	Aat	Ad Mu, He	A	Johnson et al. (1972)	1	1
Adenylate kinase	Adk	Ad Mu, He	C	Shaw and Prasad (1970)	1	0
Esterase	Est	St	A	Shaw and Prasad (1970)	1	0
Leucine aminopeptidase	Lap	Ad Mu, Ma, De, He, St, Lp	A	Wall (1968)	1	0
Malate dehydrogenase	Mdh	Ad Mu, Ma, De, He, St, Lp	C	Shaw and Prasad (1970)	0	2
Malate enzyme	Me	Ad Mu	A	Ayala et al. (1972)	0	1
Muscle protein	Mp	Ad Mu	B	Johnson et al. (1972)	1	1
Phosphoglucomutase	Pgm	Ad Mu, Ma, De, He, St, Lp	B	Shaw and Prasad (1970)	1	0
Phosphohexose isomerase	Phi	Ad Mu, Ma, De, He, St, Lp	A	Shaw and Prasad[c] (1970)	1	0
Sorbitol dehydrogenase	Sdh	Ad Mu, He	A	Shaw and Prasad (1970)	1	0
Tetrazolium oxidase	To	Ad Mu, Ma, De, He, St, Lp	ABC	Utter (1971)	0	2
				Total	8	7

[a]Tissue: Ad Mu = adductor muscle, Ma = mantle, De = demibranch, He = heart, Lp = labial palps, St = stomach.
[b]For staining procedure.
[c]Omit $MgCl_2$ from the staining mixture.

Gel buffer	15.8 mM Tris, 5 mM citric acid, 11 mM LiOH, and 42 mM boric acid, pH 8.0 (Schiff et al. 1968).
B. Stock buffer	700 mM Tris, 500 mM boric acid and 20 mM EDTA, pH 8.3
Cathode electrode buffer	dilute 1:5 with distilled H_2O
Anode electrode buffer	dilute 1:7 with distilled H_2O
Gel buffer	dilute 1:20 with distilled H_2O (Markert and Faulhaber 1965)
C. Electrode buffer	135 mM Tris, 45 mM citric acid, and 1.2 mM EDTA, pH 7.0
Gel buffer	dilute electrode buffer 1:20 with distilled H_2O (Shaw and Prasad 1970)

Filter paper wicks (3 × 8 or 3 × 14 mm, Schleicher and Schuell, No. 470) were saturated with supernatant from the homogenized samples. These wicks were inserted into a slice made across the gel about 4 cm from the cathodal end, and electrophoresis was carried out for about 15 min at 200 V for buffer systems (A) and (B), and at 150 V for buffer system (C). The wicks were then removed, an ice pack on plate-glass was placed on top of the gel, and the electrophoresis continued until a dye marker had migrated 9 cm. Upon termination of the electrophoresis, the gel was sliced horizontally into 1.6-mm slices and the cut surface stained using procedures indicated in Table 1.

Genetic premises and nomenclature — In this study a locus is considered polymorphic if the frequency of the most common allele $\leqslant 0.95$. In the absence of breeding data, the genetic explanation of polymorphic locus is based on similar patterns of variation described for enzymes of identical function with a known genetic basis reported in other organisms. Additionally, most samples had a phenotypic distribution consistent with the assumptions of Hardy-Weinberg equilibrium. Autosomal codominance appeared to be expressed in all cases of genetic variation. The heterozygote phenotypic patterns of enzyme systems reported in this study were consistent with those reported in other organisms, e.g. a dimeric structure for heterozygote phenotypes "AB" and "BC" of the Aat enzyme system (Johnson et al. 1972).

The nomenclature that has been reported previously for particular enzyme systems was retained when possible (Ayala et al. 1973; Williams et al. 1973). In Table 1, the abbreviations are given that have been used to designate each enzyme. These same abbreviations italicized are used to symbolize the genes coding for the enzymes. A hyphenated numeral is added to the symbol of the enzyme to designate each zone, and the gene coding for it when several zones of activity exist for a given enzyme.

Results and Discussion

Protein systems — Eleven enzyme systems were investigated; of these, eight were found to have loci that were polymorphic, and three were found to have loci that were completely monomorphic, as shown in Table 1. Several of the enzyme systems are apparently determined by multiple loci. Also, a few of the enzymes coded by particular loci have not been adequately resolved to permit specific analysis, but in several instances polymorphism is suspected. Three enzyme systems (Malate dehydrogenase, Malate enzyme, and Tetrazolium oxidase) were found to have monomorphic loci and will not be discussed further. The eight enzyme systems in which polymorphic gene loci were detected and scored have been interpreted in the following manner.

Aspartate aminotransferase (E.C. 2.6.1.1) — Two zones (I and II) of enzyme activity were observed for aspartate aminotransferase (Fig. 1b), which are apparently determined by at least two gene loci (*Aat*-1 and *Aat*-2). The *Aat*-1 locus, which is monomorphic, codes for the more anodal migrating enzymes located in zone I. The *Aat*-2 locus, which exhibits polymorphism, codes for the enzymes located in zone II. The different phenotypes at the *Aat*-2 locus indicated three common and two rare alleles. As can be seen in Fig. 1b, the *Aat*-2 locus exhibits on occasion two rare alleles shown in the A′B and BC′ heterozygote phenotypes. This variation appears to be identical to the polymorphism in *Ostrea lurida* described by Johnson et al. (1972).

Adenylate kinase (E.C. 2.7.4.3) — Two zones (I and II) of enzyme activity were present for

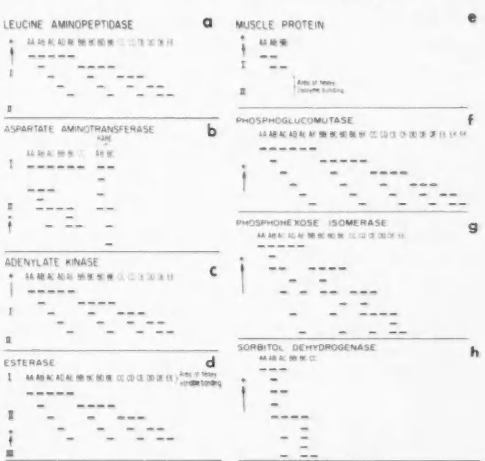

FIG. 1. The proposed phenotypic variation for eight polymorphic enzymes (Proteins) of *Crassostrea gigas*. The molecular and genetic bases for the variation of each has been described in the text. A lettering system was used to differentiate between alleles with the most anodal allele always designated at "A."

TABLE 2. Tabulation of the number of genes sampled at each locus (N), allele frequencies and standard errors, chi-square values for goodness-of-fit of observed to expected Hardy-Weinberg frequencies, and degrees of freedom for eight polymorphic loci of *C. gigas*. Also shown is a comparison of the observed and Hardy-Weinberg expected values of heterozygous individuals including their frequency and number.

Loci	N	Allele frequencies and se						χ^2	df[b]	Heterozygous individuals frequency (number)	
		A ± se	B ± se	C ± se	D ± se	E ± se	F ± se			Observed	Expected
Aat-2	262	0.29 ± 0.028	0.66 ± 0.029	0.05 ± 0.013				3.10	2	0.433 (58)	0.473 (61.9)
Adk-1	194	0.04 ± 0.013	0.16 ± 0.026	0.72 ± 0.032	0.07 ± 0.018	0.02 ± 0.009		10.15[e]	3	0.330 (32)	0.448 (43.5)[c]
Est-2	124	0.05 ± 0.019	0.28 ± 0.040	0.55 ± 0.045	0.10 ± 0.027	0.02 ± 0.013		3.62	3	0.548 (34)	0.607 (37.7)
Lap-1b	196	0.04 ± 0.014	0.11 ± 0.022	0.73 ± 0.032	0.11 ± 0.022	0.02 ± 0.009		6.94	5	0.306 (30)	0.433 (43.4)[d]
Mp-1	256	0.38 ± 0.030	0.62 ± 0.030					0.40	1	0.500 (64)	0.473 (60.5)
Pgm	196	0.10 ± 0.021	0.11 ± 0.022	0.60 ± 0.035	0.08 ± 0.019	0.10 ± 0.021	0.01 ± 0.007	8.88	8	0.541 (53)	0.604 (59.2)
Phi	196	0.00	0.06 ± 0.017	0.87 ± 0.024	0.07 ± 0.018	0.01 ± 0.005		0.34	1	0.204 (20)	0.239 (23.4)
Sdh	196	0.23 ± 0.030	0.76 ± 0.031	0.01 ± 0.007				14.11[f]	1	0.235 (23)	0.375 (36.7)[d]
	199[a]									0.210 ± 0.007[a] (314)	0.245 ± 0.008[a] (366.3)[d]

[a]Average including seven monomorphic loci.
[b]In cases when the phenotypic expectations from Hardy-Weinberg proportions were less than one, the numbers of expected and observed phenotypes were pooled with those of allele(s) of most similar electrophoretic mobility for statistical purposes (Li and Horvitz 1953) and the degrees of freedom were established accordingly.
[c]The difference between the observed and the expected number of heterozygous individuals is statistically significant, $P < 0.02$.
[d]Same, $P < 0.01$.
[e]The chi-square value for goodness-of-fit of observed to Hardy-Weinberg expectations is statistically significant, $P < 0.05$.
[f]Same, $P < 0.001$.

adenylate kinase (Fig. 1c), which are apparently determined by at least two gene loci (*Adk*-1 and *Adk*-2). The more anodal migrating enzymes located in zone I exhibit variability which indicates a polymorphism for the *Adk*-1 locus. The different phenotypes at this locus indicate five alleles. The enzymes located in zone II also show variability which indicates a polymorphism for the *Adk*-2 locus. The different phenotypes at this locus indicated four alleles. This locus never exhibited the consistent enzyme staining intensity necessary for scoring.

These results can be compared with the "killer clam," *Tridacna maxima* in which three polymorphic loci, each with four alleles, have been observed by Ayala et al. (1973). From Table 2, it can be seen that the goodness-of-fit of observed to expected frequencies is poor, reflected by a high chi-square value ($\chi^2 = 10.15_{(3)}$) and a very low probability ($0.01 < P < 0.02$) of occurrence. This statistical significance has been attributed to poor resolution which causes close migrating heterozygous phenotypes to become indistinguishable from homozygous phenotypes.

Esterase — Three zones (I, II, and III) of enzyme activity were observed with the common esterase stain (Fig. 1d) which are apparently determined by at least three gene loci (*Est*-1, *Est*-2, and *Est*-3). The more anodal migrating enzymes located in zone I stained heavily over a broad range and due to this condition the *Est*-1 locus has not been resolved. The enzymes located in zone II exhibit variability which indicates a polymorphism for the *Est*-2 locus. The different phenotypes at this locus indicated five alleles. The slowest migrating enzymes located in zone III also exhibit variability which indicates a polymorphism for the *Est*-3 locus. The different phenotypes at this locus indicated four alleles, but the locus has never been scored due to inconsistent staining intensity.

The zones of enzyme activity in *Ostrea edulis* reported by Wilkins and Mathers (1973) are similar to those described above. For example, the *EsM* locus of *O. edulis*, with two alleles, is comparable to the *Est*-2 locus of *C. gigas*, with five alleles, in that the migration distances for the enzymes appears to be the same. Wilkins and Mathers reported two polymorphic loci, each with two alleles for *O. edulis*.

Leucine aminopeptidase — Two zones (I and II) of enzyme activity have been observed for leucine aminopeptidase (Fig. 1a) which are apparently determined by three gene loci (*Lap*-1a, *Lap*-1b, and *Lap*-2). In zone I two variant enzyme forms of Lap were observed from which two polymorphic loci have been designated as *Lap*-1a (the more anodal) and *Lap*-1b. Because they are almost superimposed on each other only the *Lap*-1b locus, which codes for the slower migrating enzyme form of the two, has been analyzed. This form stains more intensely, and it is the first to develop (about 10 min). If allowed to stain longer than 10 min, the enzymes that are coded by the *Lap*-1a locus become indistinguishable from those coded by the *Lap*-1b locus. Therefore, the *Lap*-1a locus has never been amenable to further analysis. The different phenotypes at the *Lap*-1b locus indicated five alleles, while those for the *Lap*-1a locus suggested three. The enzymes located in zone II, which are thought to be coded by an additional locus (*Lap*-2), are the slowest to develop (approximately 1 h) and have not yet been clearly resolved. The two polymorphic loci (*Lap*-1a and *Lap*-1b) if separated, would be similar to the Lap activity in the soft-shelled clam *Mya arenaria*, described by Koehn and Mitton (1972). These investigators have described two polymorphic loci, each with at least three alleles in *M. arenaria*. In another pelecypod, *Tridacna maxima*, two polymorphic loci, each with four alleles, have been reported by Ayala et al. (1973).

Muscle protein — Two zones (I and II) of protein staining have been observed for muscle (Fig. 1e), which are apparently determined by at least two gene loci (*Mp*-1 and *Mp*-2). The more anodal migrating proteins located in zone I exhibit variability which indicates a polymorphism for the *Mp*-1 locus. The different phenotypes at this locus indicate two alleles. The proteins located in zone II exhibit no variability which indicates a monomorphism for the *Mp*-2 locus.

The two loci exhibited by the staining of the muscle proteins appear to be identical to those found in *Ostrea lurida* described by Johnson et al. (1972). In fact, the allele frequencies (Table 2) for the *Mp*-1 locus are identical for the two species. In our laboratory, a local population of *O. lurida* was analyzed. Results obtained from Aat and Mp did not coincide in phenotypic pattern of allele frequencies with those found by Johnson et al. (1972). This suggests that these investigators had analyzed *C. gigas* instead of *O. Lurida*; both species are endemic to the same habitat.

Phosphoglucomutase (E.C. 2.7.5.1) — One zone of enzyme activity is present for phosphoglucomutase (Fig. 1f) which is apparently determined by one gene locus (*Pgm*). The different phenotypes at this locus indicated six alleles. By comparison, in *T. maxima* two polymorphic loci,

each with four alleles, have been reported by Ayala et al. (1973).

Phosphohexose isomerase (E.C. 5.3.1.9) — One zone of enzyme activity was present for phosphohexose isomerase (Fig. 1g), which is apparently determined by one gene locus (*Phi*). The different phenotypes at this locus indicated five alleles. In addition, one rare allele has been observed on occasion which is the most cathodal migrating allele yet found. Although no "A" allele phenotypes were found in the Mud Bay population, it has been observed in other populations in Puget Sound.

The active enzyme form is consistent with that described in the European oyster, *O. edulis*, which exhibits one locus with two alleles (Wilkins and Mathers 1973), and the American eel, *Anguilla rostrata* (Williams et al. 1973).

Sorbitol dehydrogenase (E.C. 1.1.1.4) — One zone of enzyme activity is present for sorbitol dehydrogenase (Fig. 1h), which is apparently determined by one gene locus (*Sdh*). The different phenotypes at this locus indicated three alleles. Of the three alleles observed the "C" allele was extremely rare and was seen only in the "AC" and "BC" heterozygous phenotypes in very low frequency.

The active enzyme form is consistent with that described in a variety of fish species (Lin et al. 1969; Op't Hof 1969; Williams et al. 1973). Although no Sdh system has been reported for other pelecypods, Williams et al. (1973) described a polymorphic locus with three alleles in the American eel, *Anguilla rostrata*, which is similar to that found in *C. gigas*.

From Table 2, it can be seen that the goodness-of-fit of observed to expected frequencies is significantly different for the *Sdh* locus, reflected by a high χ^2 value ($\chi^2 = 14.11_{(1)}$) and a low probability ($P < 0.001$) of occurrence. This statistical significance has been attributed to the poor staining intensity of the system, although the resolution has been excellent when staining has occurred.

Mud Bay population study — The electrophoretic data obtained from the eight polymorphic loci has been tabulated in Table 2, for the Mud Bay population. The average proportion of heterozygous loci per individual was estimated by averaging over all loci the proportion of heterozygous individuals at each locus. This statistic is 21.0 ± 0.7% if the observed heterozygosities are used, and 24.5 ± 0.8% if the expected heterozygosities are used. Comparing the observed number of heterozygous individuals added over all loci (314) with the expected number (366.3) the difference is statistically significant ($\chi^2 = 9.89_{(1)}$, $P < 0.01$). This deficiency of heterozygous individuals is due for the most part to the contribution of only three loci: *Adk*-1, *Lap*-1b, and *Sdh*. At each one of these the observed frequency of heterozygous individuals is significantly smaller than the frequency expected on the assumption of Hardy-Weinberg equilibrium. For the *Adk*-1 locus ($\chi^2 = 10.15_{(3)}$, $P < 0.02$) and the *Sdh* locus ($\chi^2 = 14.11_{(1)}$, $P < 0.001$), it can be seen that the goodness-of-fit observed to expected number of individuals apparently does not fit the Hardy-Weinberg hypothesis. This statistical significance has been attributed to the incorrect scoring of the enzyme phenotypes due to the poor staining intensities as previously mentioned. The scoring of the *Lap*-1b phenotypes resulted in none of the difficulties that were observed for *Adk*-1 and *Sdh*. Since the *Lap*-1b locus ($\chi^2 = 6.94_{(5)}$, $0.2 < P < 0.3$) apparently fits the Hardy-Weinberg hypothesis, the statistically significant difference ($\chi^2 = 7.43_{(1)}$, $P < 0.01$) between the observed (30) and expected (43.4) numbers of heterozygous individuals could be attributed to some type of selective pressue experienced by this high mortality population. When the results for the *Lap*-1b locus were analyzed on a low mortality population (data not included), no significant difference was found.

Conclusion

It has been concluded from this initial study that the genetic variation encountered in *C. gigas* is worth further investigation. At present, studies are being conducted to determine if any of these polymorphic loci exhibit clinal tendencies along the Pacific coast. In addition, a study is being undertaken to determine if there is any correlation between tide levels and genetic variation. There appears to be more than adequate genetic variability on which to establish an artificial selective breeding program to attempt to reduce and/or eliminate the mortality of the Pacific oyster apparently caused by *Vibrio* species.

The genetic variability exhibited by enzyme polymorphisms can also be utilized by the oyster culture industry in other ways. Different alleles or combinations of alleles can be used as a permanent biological "tag" to identify particular strains of artificially bred oysters. These markers could potentially identify a specific hatchery stock or identify select groups that grow better under given culture conditions. Some possible selective differences were shown with one enzyme (*Lap*) in the Mud Bay population. Additionally, by analyzing populations for these enzyme poly-

morphisms, indications may be obtained concerning the genetic variations necessary for successful culture in a particular area. Thus the electrophoretic separation of enzymes/proteins demonstrated the amount and type of genetic diversity in *C. gigas* and provides a potential tool for utilization in the culture of oysters.

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Daily Pattern of Nitrogen Excretion and Oxygen Consumption of Sockeye Salmon (*Oncorhynchus nerka*) under Controlled Conditions

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BRETT, J. R., AND C. A. ZALA. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Board Can. 32: 2479–2486.

Measurements of the rate of ammonia and urea excretion of fingerling sockeye salmon (*Oncorhynchus nerka*) in fresh water were made at 2–3-hourly intervals throughout the day (average weight = 29 g; temperature = 15 C). One group of fish was fed a maintenance ration while another group was starved for 22 days. Ammonia excretion rose to a sharp peak of 35 mg N/kg per hour, 4–4½ h after the start of feeding (at 0830) and fell to a baseline level of 8.2 mg N/kg per hour between 0200 and 0800. Urea excretion remained relatively steady at a mean rate of 2.2 mg N/kg per hour throughout the day, showing no diurnal response to feeding. Starved fish showed a nitrogen excretion rate close to that for both the steady state of urea excretion and the baseline rate of ammonia excretion of the fed fish. Oxygen consumption rose to a peak of 370 mg O_2/kg per hour just before and during a 1-h feeding period, decreasing thereafter to a low of 170 mg O_2/kg per hour at 0300 h. For the starved fish this diurnal metabolic fluctuation continued from the start in a variable and diminishing form whereas nitrogen excretion showed no such response. The results are discussed in relation to hatchery observations. We conclude that for nonstressed salmon at 15 C ammonia is the chief excretory product of exogenous nitrogen metabolism.

BRETT, J. R., AND C. A. ZALA. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Board Can. 32: 2479–2486.

Nous avons mesuré le taux d'excrétion d'ammoniac et d'urée chez des alevins de la grosseur du doigt de saumon nerka (*Oncorhynchus nerka*) à intervalles de 2 à 3 h durant toute la journée (poids moyen = 29 g; température = 15 C). Un groupe de poissons reçut une ration de subsistance, alors qu'un autre groupe a été privé de nourriture pendant 22 jours. L'excrétion d'ammoniac atteint un pic abrupt de 35 mg N/kg par heure 4 à 4½ h après le début de l'alimentation (0830) et tombe à un niveau de base de 8.2 mg N/kg par heure entre 0200 et 0800. L'excrétion d'urée demeure à un niveau relativement stable, soit un taux moyen de 2.2 mg N/kg par heure. Les poissons affamés montrent un taux d'excrétion d'azote qui se rapproche à la fois de celui de l'état d'équilibre d'excrétion d'urée et du taux de base d'excrétion d'ammoniac des poissons nourris. La consommation d'oxygène atteint un pic de 370 mg O_2/kg par heure juste avant et durant une période d'alimentation d'une heure, diminuant par la suite à un minimum de 170 mg O_2/kg par heure à 0300. Ces fluctuations journalières de métabolisme se poursuivent sous une forme variable et décroissante durant la période de jeûne; l'excrétion d'azote ne présente pas une réponse journalière continue de cette nature. Nous analysons nos résultats en regard d'observations faites en pisciculture. Nous en concluons que chez des saumons non soumis à un stress, l'ammoniac est le principal produit d'excrétion du métabolisme exogène de l'azote.

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ALTHOUGH there has been extensive study on the nature of the nitrogenous excretory products of fish (Forster and Goldstein 1969) with particular attention to the general rate of exchange (Gerking 1971), there is little information on hourly rates of excretion for experimentally controlled conditions. Burrows (1964) showed that under hatchery conditons in large circular ponds, either urea or ammonia could be the dominant endproduct of protein metabolism of fingerling chinook salmon (*Oncorhynchus tshawytscha*). Irregular, large,

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daily fluctuations in the proportion of these excreta were observed. Similar results, but with less irregularity and magnitude of change, have been reported for hatchery-reared coho (*O. kisutch*) (McLean and Fraser 1974).

The following experiments were performed to determine the diurnal pattern of nitrogen excretion of young sockeye salmon (*O. nerka*) with and without feeding, under otherwise constant conditions. By studying the concomitant changes in rate of oxygen consumption, an analysis of the general exchange process associated with daily fluctuations in metabolism was undertaken.

Materials and Methods

Experimental Tanks and Operation

Two, 73-liter, oval, fiberglass tanks were used, one for fed fish, the other for starved fish. These "environmental-control" tanks were equipped with recirculating pumps, temperature regulation, water-tight tops, and water sampling ports (Brett et al. 1971). Replacement water could be flushed through continuously or intermittently, according to experimental requirements. In this case, a timing of 3 h on closed-circuit followed by 3 h on open-circuit was used for the intermittent cycle. With an average biomass of approximately 844 g per tank (mean weight of all fish at start = 28.6 + 1.2[2] g), this interval was previously established as sufficient closed-time to produce significant amounts of ammonia and urea without reducing the oxygen concentration below an adequate level of about 7–8 ppm at 15 C (70–80% saturation). By reversing the times of closed- and open-circuit during the following day, each successive 3-h period in a 24-h sequence could be accounted for by a 48-h experiment. Because a particular feeding time was involved, coverage in each hour of the 24-h cycle was important.

A week after starting, the circuit times were reduced to 2 h open and 2 h closed. Note that on open-circuit the tanks flush out solids in a matter of a few minutes; they are virtually self-cleaning. The possible production of ammonia from bacterial action was considered negligible. Iwata (1970) observed that it took 3 days after removing fish from a tank to produce a 2.2% increase in total dissolved nitrogen. Experiments were conducted for 22 days, in June, involving 12 sampling days. Excessive excitement of the fish on the first day necessitated discarding three measurements; otherwise from 28 to 32 measurements were taken of each metabolic parameter.

Ammonia and Urea Determinations

Both ammonia and urea were determined by the phenol–hypochlorite reaction of Berthelot (Henry 1967). A 100-ml sample of tank water was removed prior to and immediately after each closed cycle. Three subsamples of 10.0 ml were then analyzed for ammonia-N by adding 1.0 ml of the alkali-hypochlorite reagent, incubating at room temperature for 40 min, and recording the absorbance on a Beckman spectrophotometer. The urea in each of three 10-ml subsamples was hydrolyzed by the addition of 0.20 ml of a buffered 0.8% urease solution (for at least 2 h at 25 C) and then analyzed for urea-N as above. Ammonia and urea standards, containing 0.40 ppm ammonia-N and urea-N, respectively, were run with each determination; these agreed to within ± 5% of the mean value.

Oxygen Determinations

Separate 50-ml samples were taken in triplicate at the time of sampling for ammonia and urea. Oxygen content was determined by the unmodified Winkler method using the average of the two closest titrations. The thiosulphate solution was standardized every week using potassium bi-iodate (0.0100 $\overline{N}$).

Feeding and Food Composition

A ration of 3% body weight per day of Oregon Moist Pellets (all in dry weight weasure) was fed daily, between 0830 and 0930. For an average moisture content of the fish of 76.9%, this amounted to 6.19 dry g of food per day (= 9.10 wet g).

The composition of the diet (OMP-1) is recorded in Crawford and Law (1972); 62% of the ingredients come from fish meal. Water is added to form a total moisture content of approximately 33%. Mean protein content of the dry ingredients was 55.3%. Total digestible energy per gram wet feed was 2.4 kcal, with approximately 140 mg digestible protein per digestible kilocalorie (assuming the protein was 90% digestible [Groves 1974]).

From the above, the daily ration provided 3.06 g digestible protein per day. Because the ration was close to a maintenance level, this amount applied throughout the experiment (no significant growth occurred). On the asumption that nitrogen makes up approximately 16% of a mixed lot of proteins then 0.490 g N was digested per day by this group of fish, i.e. 553 mg N/kg per day.

Results

Nitrogen Excretion Rates — Feeding Fish

a) *Ammonia* — During the latter part of the night (after midnight) excretion of ammonia was fairly constant, at 8.2 ± 0.6 mg N/kg per hour. Following the single morning feeding, commencing at 0830, the excretion rate rose sharply to a peak of 35 mg N/kg per hour at 1300, falling rapidly thereafter in an almost exponential "die-off" to the early morning base-level, from 0200 to 0800 (Fig. 1). The overall mean rate of ammonia excretion was 14.5 mg N/kg per hour, or 349 mg N/kg per day.

[2] ± 1 SE used, except where noted otherwise.

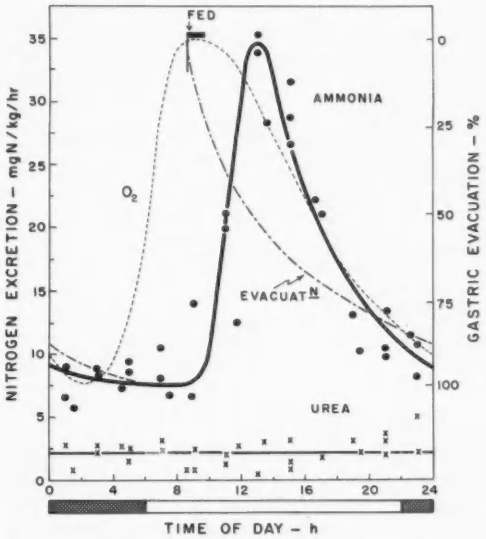

FIG. 1. Diurnal variation in rate of ammonia (●) and urea (×) excretion of fingerling sockeye salmon (*Oncorhynchus nerka*). Ration = 3% weight/day; temperature = 15 C; photoperiod = 16 h with dark period shown cross-hatched; freshwater pH = 7.0 → 6.7.) For comparison the associated oxygen consumption rate (from Fig. 2) and expected gastric evacuation rate (from Brett and Higgs 1970) have been included, scaled to the peak and baseline excretion of ammonia. Note that the oxygen consumption rates are about 10 times the nitrogen excretion rates.

b) *Urea* — In contrast to the large daily fluctuation in ammonia production, urea showed an average, continuous rate of excretion of 2.16 ± 0.20 mg N/kg per hour, independent of feeding or dark-light switching of the photoperiod control (Fig. 1). Individual values ranged from a minimum of 0.6 to a maximum of 5.0 mg N/kg per hour, but no significant trend was apparent. Total urea output was 51.4 mg N/kg per day, constituting 13% of the combined daily amonia and urea nitrogen excretion.

Nitrogen Excretion Rates — Starving Fish

a) *Ammonia* — With the cessation of feeding, on the first day, the rate of ammonia excretion fell in a manner comparable to the diurnal pulse of the 24-h cycle of the fed fish. No subsequent elevation occurred above this base-level even on the second day of starvation when the mean rate was not significantly different from that at the time of termination, 22 days later (see Fig. 3). Nor did this fixed rate (7.27 ± 0.20 mg N/kg per hour) differ significantly from the postmidnight plateau of the diurnal cycle for fed fish (*t*-test; $P > 0.5$).

b) *Urea* — Although subject to considerable variation the rate of urea excretion was distributed in an apparently random fashion around the mean of 1.91 ± 0.21 mg N/kg per hour, and was also without any change for the full 22 days. This mean rate was not significantly different from the baseline rate displayed by the fed fish (*t*-test; $P > 0.5$).

Oxygen Consumption Rates — Feeding Fish

Metabolic rates showed a large diurnal fluctuation, subject to continuous change without any lower plateau during the night, as was the case with ammonia excretion (Fig. 2). A minimum of 170 mg O_2/kg per hour occurred at 0300 rising in advance of the light (on at 0600) to reach a maximum of 370 mg O_2/kg per hour just at the start of feeding (0830). This "domed peak" of

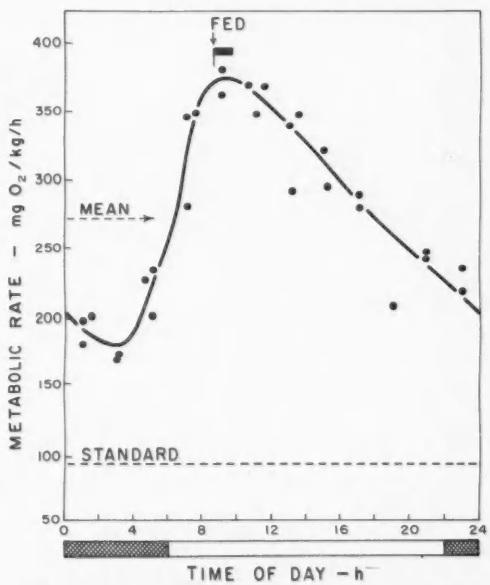

FIG. 2. Diurnal variation in metabolic rate of fingerling sockeye salmon fed a ration of 3% weight/day, from 0830 to 0930. (Temperature = 15 C; photoperiod = 16 h with dark period shown cross-hatched.) Three points shown as (+) were omitted because of excessive excitement on the first day of monitoring oxygen consumption. Data points are from cumulative observations over 22 days. Standard metabolic rate shown; active metabolic rate = 875 mg O_2/kg per hour (Brett and Glass 1973).

TABLE 1. Some daily rates of endogenous nitrogen excretion listed in order of publishing date.

Species	Weight avg or range (*g*)	Temp (*C*)	Salinity (‰)	Starvation (*days*)	Nitrogen excretion (*mg N/kg per day*)			Reference
					Ammonia	Urea	Total	
Lepomis macrochirus	50	26	fresh	3–8	—	—	154	Gerking 1955
L. macrochirus	144	26	fresh	3–8	—	—	95	Gerking 1955
Leptocottus armatus	165–391	12	30	1	41	14	64	Wood (1958)
Platichthys stellatus	310–335	12	30	1	61	9	73	Wood (1958)
Taeniotoca lateralis	360	12	30	1	14	13	30	Wood (1958)
Salmo gairdneri	129	13	fresh	6–14	75	35	136	Fromm (1963)
Lepomis macrochirus	10–100	7	fresh	3	—	—	58	Savitz (1969)
L. macrochirus	10–100	31	fresh	3	—	—	289	Savitz (1969)
Carassius auratus	1	20	fresh	7	105	—	135	Iwata (1970)
C. auratus	10	20	fresh	7	75	—	100	Iwata (1970)
S. gairdneri	50–100	13	fresh	7	—	40	160	Olson and Fromm (1971)
C. auratus	1–4	22	fresh	7	—	71	—	Olson and Fromm (1971)
L. macrochirus	70–90	24	fresh	3	—	—	114	Savitz (1971)
L. macrochirus	70–90	24	fresh	7–28	—	—	74	Savitz (1971)
S. gairdneri	900	10	fresh	4	31	—	—	Nightingale (1974)
S. gairdneri	900	15	fresh	4	55	—	—	Nightingale (1974)
S. gairdneri	900	20	fresh	4	85	—	—	Nightingale (1974)
Oncorhynchus nerka	29	15	fresh	1–22	175	46	221	Brett and Zala (this paper)

O_2-consumption was maintained through to about 1030 and then fell steadily to the low point at 0300. The mean daily metabolic rate was 274 mg O_2/kg per hour.

Oxygen Consumption Rates — Starving Fish

Although the metabolic rate of the starved fish rose in some instances during the light hours as high as that for the fed fish, there was only moderate evidence for a diurnal pattern (see Fig. 3D). Because the two tanks were adjacent, the starved fish were undoubtedly excited by the visual stimuli of the other fish being fed. Omitting the first 24 h of starvation, when excessive excitement was apparent, the mean rates for the total light and dark periods were 297 ± 14 and 170 ± 14 mg O_2/kg per hour, respectively.

A gradual decrease in daily mean metabolic rate occurred, falling from 330 ± 19 mg O_2/kg per hour on day 1 to 168 ± 17 mg O_2/kg per hour on day 21. This was accompanied by a drop in mean weight from 27.7 to 22.8 g. The lower limits of the metabolic sequence, represented by the mean of the night observations from day 6 to day 22 (155 ± 20 mg O_2/kg per hour), were only 15 mg O_2/kg per hour below the lowest level of the diurnal pulse of the fed fish. Indeed, on day 6 the nighttime average was 169 mg O_2/kg per hour, approximately equal to the minimum level at 0300 for fed fish. This would indicate that any metabolic demand associated with food processing was satisfied approximately 18 h after feeding.

Discussion

Nitrogen Excretion — Exogenous and Endogenous Fractions

The dynamic state of tissue proteins, involving catabolic and anabolic processes in an open system, led to the well-recognized concept of a metabolic pool of amino acids (see discussion in: Cowey and Sargent 1972). Exogenous and endogenous sources are common to this pool. To separate the relative contributions, endogenous nitrogen excretion was defined for domestic animals as "the lowest level of N-excretion attained after an empirically defined time interval on a low nitrogen but otherwise complete diet" (Brody 1945). This maintains the normal energy requirements of the animal without involving it in any serious nutritional stress from complete lack of a nitrogen source, i.e. just maintaining the status quo without sacrificing body protein. In accordance with the need for meeting energy expenditure, Savitz (1969, 1971) force-fed just enough glucose to bluegill (*Lepomis macrochirus*) to meet their estimated maintenance metabolism (following the method of Gerking 1955).

Records of N-excretion of starved animals provide a near approximation of the endogenous fraction, particularly for cold-blooded vertebrates that normally endure prolonged periods of starvation. Error may be minimized by extrapolating back to zero time (postdigestive state), where it is obvious that any decay in maintenance metabolism can be considered as insignificant. In the case of the sockeye there was no apparent decline over the 22-day starvation period. The rate of endogenous loss from the nitrogen pool was determined as 9.2 mg N/kg per hour (total of ammonia-N and urea-N).

This amount is not different from the sum of the urea-N and baseline ammonia-N (applied over 24 h) of the fed fish (Fig. 1). The fraction in excess of this for the fed fish represents the exogenous nitrogen excretion, which amounted to 7.4 mg N/kg per hour. It consisted entirely of ammonia.

Gerking (1955) demonstrated a weight-dependent relation for endogenous N-excretion (approx. $W^{0.54}$), following the well-known relation of decreasing rate of metabolism with increasing size. It is undoubtedly important to take size into consideration when comparing various endogenous rates among fishes; however, some doubt has been cast on the correct exponent (see: Savitz 1969; Iwata 1970), and it can be expected that considerable species variation will occur. From the compilation of some of the existing literature in Table 1 it is apparent that the sockeye displayed one of the highest rates of endogenous excretion so far recorded, and that ammonia-N predominated over all other species. A contributing factor to this relatively high rate in sockeye is the fact that the fish were swimming in an average water velocity of about 10–12 cm/s (0.7 L/s) and had a daily metabolic rate equal to about 2.3 times standard metabolism.

Nitrogen Budget

The foregoing considerations have not dealt with the total nitrogen relation, which includes the rates of intake, storage (growth), and fecal loss. Since there was no significant change in body weight, the balance of the daily food input of 490 mg N (= 553 mg N/kg per day) was likely excreted as feces. By difference, this amounted to 88 mg N, or 18% of the assumed "digestible" protein.

This circumstance undoubtedly accounts for the lack of any growth, which was expected to be about 1%/day rather than at a maintenance level.

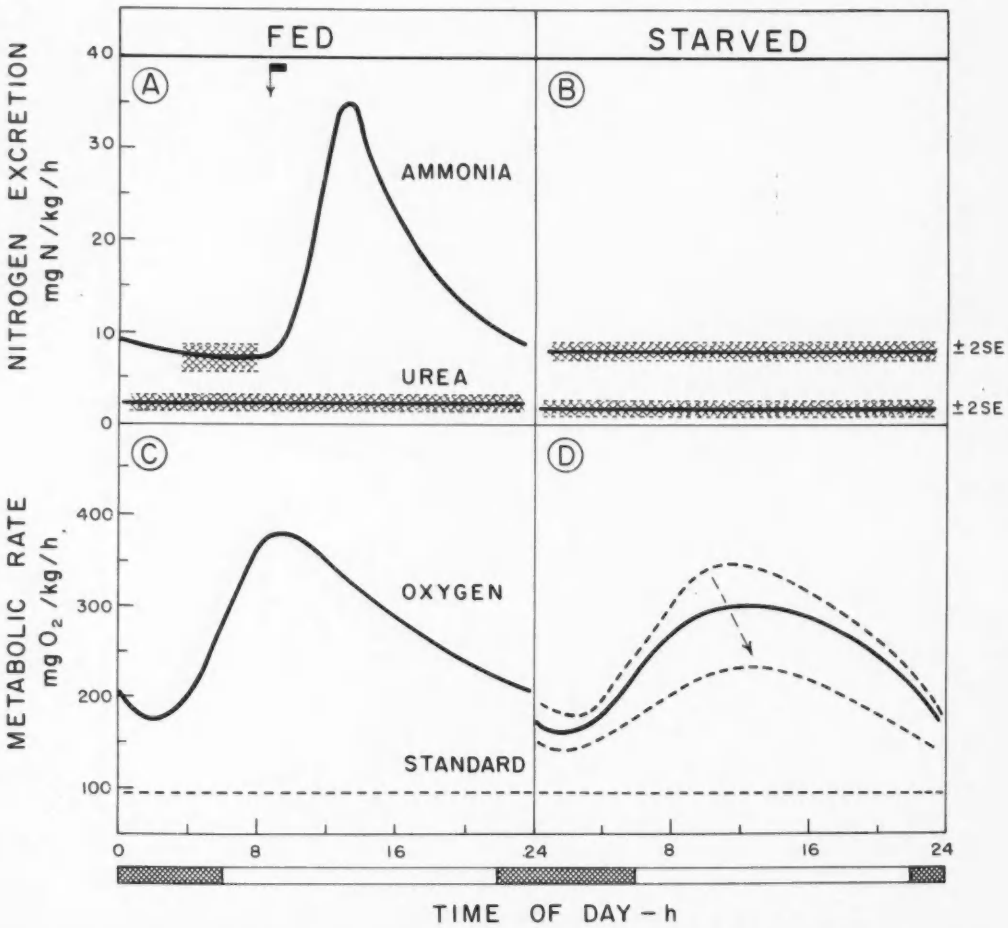

FIG. 3. Pattern of change in rates of nitrogen excretion (A, B) and oxygen consumption (C, D) of sockeye salmon when switched from a single daily feeding to starving for 22 days. Limits of ±2 SE are shown for data points on ammonia and urea excretion. The metabolic pulse for starved fish (D) diminished with deprivation time and became diffuse; rates during light hours were significantly higher than during dark hours (cross-hatched). The average metabolic trend isportrayed (solid line) with broken lines indicating the general decrease from start to finish (schematic).

No explanation for this phenomenon can be offered except to speculate on a possible over-extended shelf life of the food causing deterioration in vitamin content and or possible slight rancidity; it seems unlikely that normal biological variability would account for this degree of difference. No food was left uneaten.

DIURNAL PATTERN OF RATES OF EXCRETION AND METABOLISM

a) *Feeding fish* — By superimposing a scaled plot of the metabolic rate and gastric evacuation rate over that for ammonia excretion the time sequence of each system can be readily compared (Fig. 1). Metabolic rate rose prior to and had already reached a maximum at the start of feeding. Ammonia excretion remained at the baseline level until feeding had started, rising to a peak 4–4½ h after feeding commenced. The two rates of ascent were almost parallel. Metabolic rate fell prior to any decline in ammonia excretion, but a general parallelism existed that was also in harmony with gastric evacuation rate.

At the time of peak ammonia excretion the stomach would be close to half empty. Deamination of amino acids in the blood is undoubtedly proceeding at its highest rate, coupled with some ill-defined metabolic contribution. The large pulse in ammonia excretion appears to be entirely a product of food intake (exogenous), and not of metabolic rate. An examination of the starvation curves (below) supports this.

The maximum metabolic rate at feeding time (375 mg O_2/kg per hour) is approximately 43% of the expected active metabolic rate (approximately 875 mg O_2/kg — Brett and Glass 1973). As noted, the daily average of 274 mg O_2/kg per hour was about 3 times the expected standard rate (95 mg O_2/kg per hour).

b) *Starving fish* — Although somewhat diffuse, a declining diurnal pulse in the metabolic rate of the starved fish was not accompanied by any detectable pulse in either NH_3 or urea excretion (Fig. 3). This is rather remarkable because metabolic use of protein as an energy source is well known in fish. It would appear as though maintenance levels of energy requirement were supported by protein metabolism, while higher rates drew on such carbohydrate and fat reserves as were readily available.

Comparison with Hatchery Records

The large fluctuations in the production of urea from hatchery fish (chinook and coho), and the presence of undetectible ammonia[3] on occasion is in marked contrast to the circumstances observed under the highly controlled conditions of the laboratory tests on sockeye. Burrows (1964) observed that low stocking density in raceways (1.64 lb/gpm[4]) coupled with low temperatures (8 C) resulted in predominance of urea, whereas high density (8.16 lb/gpm) associated with relatively high temperatures (13 C) resulted in predominance of ammonia. When ammonia did predominate it tended to occur over 14 h of the day with a peak at about 1600 h. The total nitrogen from urea and ammonia was fairly uniform over the full 24 h. Feeding is normally spread out over an 8-h day in hatcheries (not recorded in Burrows 1964).

The extensive hatchery studies of McLean and Fraser (1974) on the nitrogen excretion of coho salmon tend to support Burrows' (1964) observations on chinook salmon. Ammonia-N accounted for over 60% of the total nitrogen excreted, rising to over 90% on some days. Relatively high temperatures, major disturbances, and high-loading density tended to be associated with high rates of ammonia excretion. With fish of 12–15 g weight, on a dawn to dusk feeding regime, a daily pulse in ammonia excretion occurred, the rate rising with the commencement of feeding reaching a peak about 9 h later (1500–1700). There was a high correlation with daily fluctuations in temperature, particularly peak temperatures ($r = 0.90$). However, on one occasion when fish were not feeding ammonia production did not rise; this indicates the likely confounding of feeding and digestion processes with daily temperature fluctuations resulting in a spurious correlation. The average minimum (9.3 mg N/kg per hour) and maximum (40 mg N/kg per hour) rates of ammonia excretion are comparable with those recorded for the sockeye. The major differences occur in the rapid rate of diurnal increase in ammonia production following feeding in sockeye (4 h to peak vs. 11 h in coho), and the continuously low, relatively stable excretion of urea in contrast to a higher, more variable rate in hatchery coho. Although differences in conditions were great, it is unlikely that there was considerable endogenous ammonia in the sockeye excretion peak drawn from the nitrogen pool rather than the dietary protein of that day's meal; also, the high stability of the environmental conditions resulted in greater stability of urea production.

It is apparent that systematic records of the concentration of ammonia, urea, and oxygen in the effluent water of hatcheries, in relation to feeding, loading density, and temperature should provide improved insight on the circumstances that promote health and good growth in cultured stocks.

[3]Ammonia would not be lost to any extent from such an open system because less than 1% is unionized at pH of 7.6 or lower, for the temperature involved.

[4]gpm = gallons per minute.

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Culture Studies on the Effects from Borate Pollution on the Growth of Marine Phytoplankters

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ANTIA, N. J., AND J. Y. CHENG. 1975. Culture studies on the effects from borate pollution on the growth of marine phytoplankters. J. Fish. Res. Board Can. 32: 2487–2494.

The autotrophic growth of 19 species of marine phytoplankters, from 10 classes of algae, was tested on axenic cultures with boric acid additions of 0–100 mg/liter B to a nutrient-enriched, pH-controlled seawater medium. All the growth rates were virtually unaffected by the incorporation of 5–10 mg/liter B, while 26% of the species were strongly inhibited by 50 mg/liter B and this proportion was increased to 63% species inhibited at 100 mg/liter B. Several species required prolonged periods of adaptation before exponential growth with 50–100 mg/liter B. Both the adaptation period and the degree of inhibition were gradually mitigated on sequential transfer of certain species from lower to higher boron concentration. Such sequential transfer tests showed that the majority of initially inhibited species could recover good growth at 50 mg/liter B but not at 100 mg/liter B, which concentration appeared to be lethal to 37% of the species tested. These results predict that, in the absence of stress from nutrient deficiency and pH adversity, inorganic borate pollution would be facilely tolerated by phytoplankton up to 10 mg/liter B; higher borate concentrations up to 100 mg/liter B are expected to cause species redistribution tending to favor growth of some forms by suppressing that of others.

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Nous avons étudié la croissance autotrophe de 19 espèces de phytoplanctontes marins, appartenent à 10 classes d'algues, en cultures axéniques avec additions d'acide borique de 0–100 mg/litre de B à un milieu d'eau de mer à pH contrôlé et enrichi d'éléments nutritifs. Le rythme de croissance de toutes les espèces demeure virtuellement inchangé par l'addition de 5–10 mg/litre de B, alors que 26% des espèces sont fortement inhibées par 50 mg/litre de B. Ce pourcentage passe à 63% avec 100 mg/litre de B. Plusieurs espèces requièrent une longue période d'adaptation avant la croissance expérimentale avec 50–100 mg/litre de B. La période d'adaptation et le degré d'inhibition sont tous deux graduellement atténuée par le transfert de certaines espèces d'une faible teneur en bore à une teneur plus forte. Les essais impliquant de tels transferts démontrent que la majorité des espèces initialement inhibées peuvent retrouver une bonne croissance à 50 mg/litre de B, mais non à 100 mg/litre de B, cette dernière concentration semblant létale pour 37% des espèces étudiées. Ces résultats permettent de prédire que, en l'absence de stress causé par une déficience alimentaire et un pH adverse, la pollution par borate inorganique peut facilement être tolérée par le phytoplancton jusqu'à une concentration de 10 mg/litre de B. On peut s'attendre que de plus fortes concentrations, jusqu'à 100 mg/litre de B, causeront redistribution des espèces, tendant à favoriser la croissance de certaines formes en retardant celle d'autres formes.

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BORATE (or boric acid) occurs naturally in sea water at levels of 4.5–5.5 mg/liter B varying with the salinity or chlorinity content of sea water (Noakes and Hood 1961; Gassaway 1967). Approximately 76% of total inorganic boron in sea water occurs as undissociated boric acid [$B(OH)_3$], with the remainder identified as the borate ion $B(OH)_4^-$; 44% of this ion appears to be complexed with sodium, magnesium, and calcium (Byrne and Kester 1974). Other evidence suggests additional complexation of borate with ferric ions (Kester and Byrne 1972) and polyhydroxylated organic compounds (Noakes and Hood 1968). Like silicate, borate is known to be an essential micronutrient for growth of diatoms (Lewin 1965, 1966), and it appears that a chemical combination of both micronutrients in the form of silicoborate may be required by certain diatoms (Werner 1969).

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Recent evidence for accumulation of deleterious zinc concentrations from industrial effluents by marine fauna (e.g. Eisler and Gardner 1973; Ichikawa and Ohno 1974; Ratkowsky et al. 1974) has obliged certain pulp and paper mills in coastal areas of British Columbia to replace zinc dithionite (normally used as the groundwood bleaching or brightening agent) by sodium dithionite. The latter reagent is produced in situ at the mill by the Borol process reaction shown by Equation 1, with concomitant formation of sodium metaborate as a by-product, which is disposed of with the mill effluent. In aqueous solution, the metaborate ion is rapidly converted to the borate anion and the weakly dissociated boric acid by the sequential reactions shown by Equations 2 and 3.

$$NaBH_4 + 8\ NaOH + 8\ SO_2 \rightarrow 4\ Na_2S_2O_4 + NaBO_2 + 6\ H_2O \quad (1)$$

$$BO_2^- + 2\ H_2O \rightarrow B(OH)_4^- \quad (2)$$

$$B(OH)_4^- + H_3O^+ \leftrightarrows B(OH)_3 + 2\ H_2O \quad (3)$$

The net chemical effect of pulp-mill metaborate-pollution is thus to increase the natural borate concentration of sea water. This increase is at present estimated as generally slight (arising from ca. 0.7–0.9 ppm boron in mill effluent, J. A. J. Thompson [unpublished data], but it is feared that continuous long-term pollution may progressively elevate borate in coastal areas to levels deleterious to one or more forms of marine life. In this connection, it was desirable to investigate the degree of toxicity from elevated boric acid concentration to marine phytoplankton.

In order to establish that the effects from borate concentration would not be compounded by the multiplicity of uncontrollable physical, chemical, and biological parameters prevalent in the marine environment, we have chosen to conduct this study with axenic cultures of phytoplankters from a wide cross section of algal taxonomic divisions and to measure growth rates with a range of borate concentrations under constant culture conditions ensuring a) sufficiency of nutrients and light for growth, and b) maintenance of pH near that of sea water. The likelihood of slow or gradual adaptation of phytoplankton to increasing borate concentration was also considered in this investigation, and exploratory tests were made on the possibility of neutralizing the toxicity from high borate levels by the simultaneous incorporation of a polyhydroxy organic compound capable of complexing with borate ions. The comprehensive results of this investigation are reported below.

Materials and Methods

Phytoplankters

Axenic cultures of 19 marine species (listed in Table 2) were chosen at random from 10 classes of algae. Unless otherwise stated, their strain (or clone) symbols and sources were the same as previously reported (Antia and Cheng 1970). The presently designated *Emiliania huxleyi* and *Thalassiosira pseudonana* are nomenclatural revisions, due to Hay et al. (1967) and Hasle and Heimdal (1970), of the earlier reported *Coccolithus huxleyi* and *Cyclotella nana*, respectively. The species named *Bellerochea polymorpha* corresponds to Guillard's tropical clone 675 D or 675-d isolated off the coast of Surinam (Carpenter and Guillard 1971; Hargraves and Guillard 1974), while *Monallantus salina* corresponds to Maestrini's isolate from the Gulf of Marseille (Berland et al. 1970); both these strains were obtained in axenic culture from their respective isolators.

It is pointed out that, whereas *Nannochloris oculata* was previously placed in the Chlorophyceae (Droop 1955) and *Monallantus salina* in the Xanthophyceae (Berland et al. 1970), we have transferred them to the Eustigmatophyceae on the basis of more recent observations (Antia et al. 1975).

Test Methods

The tests were made in seawater medium of composition shown in Table 1, with analytical reagent grade boric acid purchased commercially and used without further purification. Suitable aliquots (4 ml) of the growth medium, containing 0, 5, 10, 50, 100 mg/liter B, in 8-ml capacity screw-capped culture tubes (chosen to be optically clear) were inoculated with 0.2-ml aliquots of a stock culture (generally 10–15 days old) and incubated stationary in continuous, cool-white fluorescent light at 16–20 C; the tubes were placed inclined at an angle of 22–24° from the horizontal plane and the illumination intensity reaching them, from lights placed horizontally above them, was estimated at ca. 100–150 foot candles. Growth measurements were made, at intervals of 2–4 days, on intact cultures by determining the optical density at 600 nm (Bausch and Lomb Spectronic 20) after vortex-mixing. Exponential growth rates were calculated, as previously described (Cheng and Antia 1970), from the plots of turbidity increase with incubation time (Fig. 1) and expressed as the percentage of corresponding growth obtained in each control taken without borate. Axenic conditions were ensured by using standard aseptic techniques and by taking periodic sterility checks.

A similar procedure was used for the sequential transfer tests of adaptation to increasing borate concentration, using for inoculum the mature growth obtained on the highest borate concentration initially tolerated. When required as a borate-complexing agent, glycerol was incorporated simultaneously with boric acid into the growth medium before inoculation; the glycerol concentration was calculated to be equivalent to that of the boron used.

TABLE 1. Composition of phytoplankter growth medium used for boron toxicity studies. Boric acid concentrations of 0, 5, 10, 50, 100 mg/liter B were incorporated into the growth medium, known to contain 3.65 (± 0.20) mg/liter endogenous B*. This latter B was contributed to the medium by the natural sea water used, which was found to contain 4.50 (± 0.10) mg/liter B*.

KNO_3	250 mg (2500 μmole)[a]
$NaH_2PO_4 \cdot H_2O$	34.5 mg (250 μmole)[a]
$Na_2SiO_3 \cdot 9H_2O$	84.0 mg (300 μmole)[a]
Vitamins:	
Thiamine·HCl	500 μg (1.48 μmole)
Biotin	1 μg (0.0041 μmole)
B_{12}	2 μg (0.00134 μmole)
Trace-metal ions (chelated)[a]:	
$Na_2 \cdot EDTA \cdot 2H_2O$	8.1 mg (21.8 μmole)
$FeCl_3 \cdot 6H_2O$	2.7 mg (10.0 μmole)
$MnSO_4 \cdot 4H_2O$	1.125 mg (5.0 μmole)
$ZnSO_4 \cdot 7H_2O$	0.575 mg (2.0 μmole)
$Na_2MoO_4 \cdot 2H_2O$	0.243 mg (1.0 μmole)
$CuSO_4 \cdot 5H_2O$	0.025 mg (0.1 μmole)
$CoSO_4 \cdot 7H_2O$	0.014 mg (0.05 μmole)
Buffer:	
Tris·HCl {41.3 mM, pH 6.8–6.9 before autoclaving}[b]	200 ml (1 g or 8.3 mmol Tris)
Sea water, Pacific Ocean, salinity 33‰[c]	To 1 liter

*The boron content was determined by the method of Uppström (1968).

[a]pH of stock solutions of these components was adjusted to 7.6–7.8 with aqueous HCl or NaOH before incorporation with the rest of the medium.

[b]This gives a pH of 7.6–8.0 in the medium after autoclaving (15 min at 120 C).

[c]Surface water from the open ocean Station "P" at 50°N, 145°W (McAllister et al. 1960); this water was aged several months in the laboratory, at ambient room temperature, before use.

Results

It was recognized from the various shades of borate-concentration effects on the different species (examples shown in Fig. 1) that both the growth rate and the preexponential lag period must be taken into account in assessing the ecological consequences of sudden or persistent pollution. Based on these considerations, the results of the survey made with first exposure of the algae to the full range of borate concentration are summarized in Table 2. These results establish that levels of 5–10 mg/liter B have no inhibitory effect on the growth of any of the species; in fact the 10-mg level appeared to stimulate the growth of a few. Moreover, it appeared that critical concentrations in the range 10–50 mg/liter B may favor prolonged survival of peak populations of certain diatoms after cessation of growth; this phenomenon was observed with *Bellerochea polymorpha* at 10 mg/liter B and with *Skeletonema costatum* at 50 mg/liter B (Fig. 1). However, in general, the 50-mg level depressed the growth rate of about half the total number of species to the extent of 30–70%, while the 100-mg level was strongly inhibitory towards 63% of the species and appeared to be lethal to two thirds of this percentage. Several species showed prolonged preexponential lag periods on the higher borate concentrations (Table 2). With 100 mg/liter B this adaptation period was most striking for both the eustigmatophytes in that growth was imperceptible until about 34–36 days after inoculation (Fig. 1 and 2). A chlorophyte (*D. tertiolecta*), a diatom (*T. pseudonana*), and a cyanophyte (*A. marina*) were exceptional in showing neither significant inhibition nor adaptation lag on first exposure to 100 mg/liter B (Table 2, Fig. 1).

The question of lethality or impaired growth

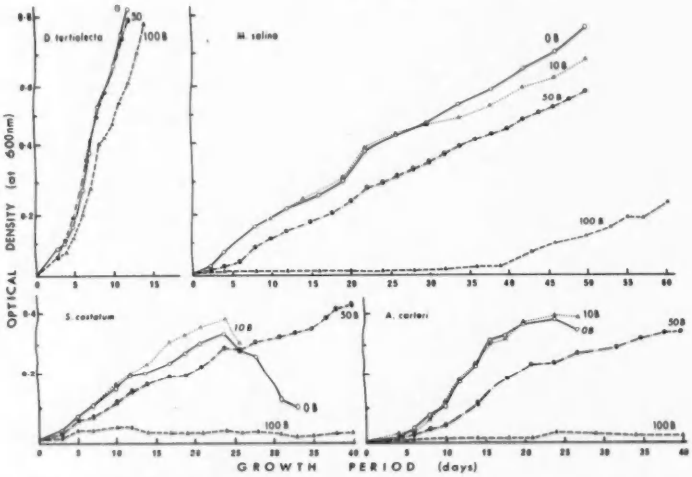

FIG. 1. Growth of four phytoplankter species from first exposure to boric acid — B (mg/liter) concentrations as shown: ○———○ 0 B; △·····△ 10 B; ●—·—● 50 B; ▲- - -▲ 100 B.

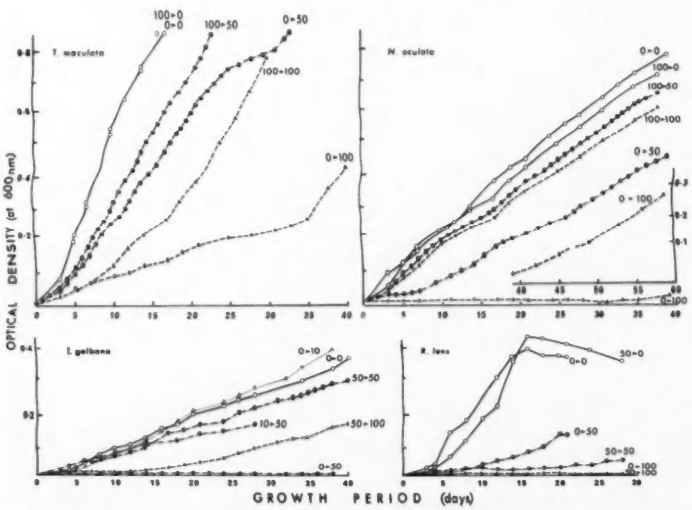

FIG. 2. Examples of growth effects following sequential transfer of four phytoplankter species from the mature growth obtained at definite boron concentrations in the range 10–100 mg/liter to similar and other boron concentrations within the same range. Corresponding transfers to controls without added boron (0 mg/liter) are included, where required. For B concentration key, see Fig. 1 legend. Each sequential transfer is shown by an arrow leading from the B concentration providing the inoculum to that at which the depicted growth was observed. All boron concentrations are shown by numerals only.

TABLE 2. Summary of boron concentration effects on phytoplankter growth.

Species tested	% Growth rate[a] (*a*) and Preexponential lag period (*b* days) from added boric acid concentration (mg/liter B) of							
	0–5		10		50		100	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Chlorophyceae								
Dunaliella tertiolecta	100	4	100	4	100	4	82	4
Prasinophyceae								
Tetraselmis maculata	100	6	100	6	42	7	43	35
Haptophyceae								
Emiliania huxleyi	100	10	—	—	32	19	0	∞
Isochrysis galbana	100	4	100	4	0	∞[b]	0	∞
Chrysophyceae								
Monochrysis lutheri	100	7	105	7	62	20	33	39
Eustigmatophyceae								
Nannochloris oculata	100	3	96	3	85	25	86	49
Monallantus salina	100	8	88	8	77	10	65	40
Bacillariophyceae (diatoms)								
Phaeodactylum tricornutum	100	4	96	4	46	4	46	4
Thalassiosira fluviatilis	100	4	100	4	100	4	0	∞
Thalassiosira pseudonana	100	4	100	4	100	4	100	4
Cyclotella cryptica	100	7	100	7	86	7	0	∞
Skeletonema costatum	100	5	129	4	79	5	0	∞
Bellerochea polymorpha	100	4	100	4	92	4	46	4
Cryptophyceae								
Chroomonas salina	100	5	100	5	71	7	0	∞
Rhodomonas lens	100	5	125	6	31	9	0	∞
Dinophyceae								
Amphidinium carteri	100	10	100	10	72	13	0	∞
Rhodophyceae								
Porphyridium cruentum	100	7	100	7	105	8	82	15
Cyanophyceae								
Agmenellum quadruplicatum	100	4	100	4	100	4	100	33
Anacystis marina	100	3	123	3	118	3	95	5

[a]Maximum (exponential) growth rate expressed as percent of that obtained in control with no added boron.
[b]Symbol denoting infinite period.

from the shock of sudden exposure to high borate concentration was next considered from the viewpoint that the more susceptible species might show greater tolerance through gradual exposure to increasing borate concentration. This was indeed observed with many species on sequential transfer from growth at one borate level to the same and next higher level (Table 3). Such gradual adaptation was best witnessed with *Isochrysis galbana*, which could not tolerate 50–100 mg/liter B on first exposure but was induced to accept such borate levels with continually improving growth performance on sequential transfers (Fig. 2). On the other hand, the lethality of 100 mg/liter B to several species was not removed after adaptation to 50 mg/liter B (Table 3). The successive transfer tests on 50 or 100 mg/liter B showed a general tendency to shortening of the prolonged preexponential lag period obtained from several species on first exposure to these borate concentrations, but this trend was not always accompanied by improvement in growth rate (Table 3). *Amphidinium carteri* continued to show impaired growth on second transfer at the 50-mg level, while *Rhodomonas lens* could barely tolerate this level on first exposure and succumbed to its toxicity on the next transfer (Fig. 2).

Since borate is known to complex or chelate with a variety of polyhydroxylated organic compounds including phenolics and carbohydrates (Hakoila et al. 1972; Pettersson and Theander 1973), it was desirable to know whether such complexation with a polyol might neutralize the toxicity shown by 100 mg/liter B towards the more susceptible phytoplankters. Glycerol was chosen as the complexing agent, since it was known to be beneficial to phytoplankter growth

TABLE 3. *Phytoplankter growth adaptation on sequential transfer to increasing boron concentration.*

Species tested	Transfer*	% Growth rate (*a*) and Preexponential lag period (*b* days) from added boric acid concentration (mg/liter B) of							
		0		10		50		100	
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
T. maculata	1st	100	6	—	—	—	—	43	35
	2nd	100	5	—	—	—	—	79	24
N. oculata	1st	100	3	—	—	—	—	86	49
	2nd	100	0	—	—	—	—	86	2
E. huxleyi	1st	100	10	—	—	32	19	0	∞**
	2nd	100	3	—	—	63	7	0	∞
	3rd	100	7	—	—	108	11	0	∞
I. galbana	1st	100	4	100	4	0	∞	0	∞
	2nd	100	4	100	4	67	4	—	—
	3rd	100	4	—	—	90	4	70	24
M. salina	1st	100	8	—	—	—	—	65	40
	2nd	100	0	—	—	—	—	99	10
C. cryptica	1st	100	7	—	—	86	7	0	∞
	2nd	100	7	—	—	85	11	0	∞
C. salina	1st	100	5	—	—	71	7	0	∞
	2nd	100	4	—	—	97	4	0	∞
R. lens	1st	100	5	—	—	31	9	0	∞
	2nd	100	6	—	—	0	∞	0	∞
A. carteri	1st	100	10	—	—	72	13	0	∞
	2nd	100	5	—	—	60	6	0	∞
A. quadruplicatum	1st	100	4	—	—	—	—	100	33
	2nd	100	4	—	—	—	—	90	4

*In each case, the 1st transfer was made from a stock culture containing no added B; subsequent transfers were made from the highest B concentration showing significant growth to similar and higher B concentrations as well as to a control with no added B.

**Symbol denoting infinite period.

TABLE 4. Tests of phytoplankter growth in combined presence of borate and glycerol.

Species tested	Exponential growth rates[a] from presence/absence of added boric acid (B mg/liter) and/or glycerol (Glyc. mmole/liter)			
B:	0	0	100	100[b]
Glyc.:	0	10	0	10[b]
E. huxleyi	0.012	0.013	0	0
C. cryptica	0.014	0.015	0	0
C. salina	0.041	0.053	0	0

[a]Expressed arbitrarily as daily rate of change in culture optical density during exponential phase of growth.

[b]Since 100 mg/liter B corresponds to 9.26 mmole/liter borate, it is pointed out that the molar ratio borate/glycerol is 0.93.

(Cheng and Antia 1970). Such tests were made by incorporating equivalent glycerol into the growth medium at the same time as borate and taking corresponding controls with glycerol only. The results (Table 4) showed that glycerol alone gave good growth but it failed to mitigate the borate toxicity in all cases tested. Since we have not directly verified the complexation of borate with glycerol in the test medium, this failure could result from inadequate complex formation or from the complex itself being toxic to the phytoplankters. Further experimentation to resolve this question was considered beyond the scope of this investigation.

Conclusions

At the outset, it must be pointed out that the results of this study may be ecologically extrapolated only to *eutrophic waters receiving borate*

additions without drastic pH change. Subject to this condition, our results anticipate that boron pollution up to 10 mg/liter from pulpmill effluent would be readily tolerated by marine phytoplankton and would cause little, if any, destabilization of natural communities. This prediction assures that there is no recognizable hazard to coastal phytoplankton populations from the tenfold lower borate concentrations currently released by the mills.

Our results also infer that higher boron additions up to 100 mg/liter would cause species redistribution tending to favor growth of the more tolerant types of microalgae by suppressing that of the sensitive forms. If such boron concentrations are gradually accumulated from long-term pollution, it appears from our tests that most phytoplankters have the potential to increase their tolerance levels and resume normal growth. In the event of a sudden surge of boron concentration from an accidental "spill" we anticipate substantial species redistribution but not necessarily a serious loss of long-range primary production. However, such species redistribution may require a change in the feeding habits of the next link in the marine food-chain, whose capacity for such adaptation remains unknown. In this connection, it was of interest to consider remedial measures to counter the toxic effects from a sudden borate "spill" by incorporating an innocuous borate-complexing agent into the affected water system, but it appears from our preliminary tests that glycerol may not serve this purpose.

The literature on the problem of boron pollution of freshwater systems has been well reviewed by Waggott (1969); interestingly, this author's investigation also concludes that the present boron levels in British waters do not present an immediate toxicity problem. Apart from our study, we are aware of only one published report on borate concentration effects towards microalgae. Forsberg et al. (1967), investigating the influence of synthetic detergents on growth of freshwater species, found that boron concentrations in the range 10–200 μg/liter retarded the growth rate of some species but accelerated that of others; as these concentrations are more than one order of magnitude below the endogenous boron level of sea water, there is no valid basis for comparison with our results.

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Toxicity of 30-Minute Exposures of Residual Chlorine to the Copepods *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*[1]

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LATIMER, D. L., A. S. BROOKS, AND A. M. BEETON. 1975. Toxicity of 30-minute exposures of residual chlorine to the copepods *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*. J. Fish. Res. Board Can. 32: 2495–2501.

The intermittent chlorination of power plant cooling waters often results in the appearance of residual chlorine in the receiving waters. Laboratory bioassays were conducted at 5, 10, 15, and 20 C to determine the toxicity of residual chlorine exposures of 30 min to the copepods *Limnocalanus macrurus* and *Cyclops bicuspidatus thomasi*. The 30-min TL-50 was 1.54 mg/liter for *L. macrurus* at both 5 and 10 C. For *C. b. thomasi*, the 30-min TL-50 values for exposures at 10, 15, and 20 C were 14.68, 15.61, and 5.76 mg/liter, respectively. The concentrations of residual chlorine causing 5% mortality (30-min TL-5) were used to predict "safe" levels for 30-min exposures. The predicted "safe" concentrations were 0.9 mg/liter for *L. macrurus* and 0.5 mg/liter for *C. b. thomasi*. These concentrations roughly coincide with the maximal chlorine levels characteristic of most power plant effluents.

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La chlorination intermittente des eaux de refroidissement de centrales électriques a souvent pour effet l'apparition de chlore résiduaire dans les eaux qui les reçoivent. Nous avons fait des analyses biologiques en laboratoire à 5, 10, 15 et 20 C dans le but de déterminer les niveaux toxiques (NT) d'expositions de 30 mn au chlore résiduaire chez *Limnocalanus macrurus* et *Cyclops bicuspidatus thomasi*. Le NT-50 après 30 mn est de 1.54 mg/litre pour *L. macrurus* tant à 5 qu'à 10 C. Pour *C. b. thomasi*, les valeurs de NT-50 après 30 mn à 10, 15 et 20 C sont respectivement de 14.68, 15.61 et 5.76 mg/litre. Les concentrations de chlore résiduaire causant une mortalité de 5% (NT-5 après 30 mn) ont servi à prédire les niveaux « sûrs » d'expositions de 30 mn. Ils sont de 0.9 mg/litre pour *L. macrurus* et de 0.5 mg/litre pour *C. b. thomasi*. Ces concentrations coincident à peu près avec les niveaux maximums caractéristiques des effluents de la plupart des centrales électriques.

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CHLORINE is often added to power plant cooling water to prevent the formation of algal and bacterial slimes on condenser heat-exchange surfaces. Procedures generally involve shore chlorination periods, which often result in the brief appearance of residual chlorine in the waters receiving the plant effluent. The toxicity of residual chlorine to aquatic life is well documented, and has been reviewed extensively (Brungs 1973; Becker and Thatcher 1973). Most laboratory-derived information on chlorine toxicity has involved exposure periods of from 4 to 7 days while the typical power plant may chlorinate in short "slug" doses (Draley 1972). Further, fish have been used almost exclusively as test organisms in toxicity studies, and little information is available concerning the effects of chlorine on invertebrates.

Hale (1930) reported that *Cyclops* sp. was killed by a 20-min exposure to 2–3 mg/liter residual chlorine in a public water supply. Heinle (1969) attributed the high mortality rate of the marine copepod *Acartia tonsa* in a power plant effluent to the chlorination of the cooling water, correlating the apparent periodicity in the mortality rate with the chlorination schedule. Arthur and Eaton (1971), in a laboratory study, found that the 96-h TL-50 for the amphipod *Gammarus pseudolimnaeus* was 0.220 mg/liter, and that re-

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production was reduced at concentrations above 0.0034 mg/liter. Grossnickle (1974) reported that the 4-h and 1-h TL-50 values for the rotifer *Keratella cochlearis* were 0.027 and 0.032 mg/liter respectively. No definitive information could be found regarding the toxicity of chlorine to invertebrates for exposure periods as short as 30 min.

The objective of the present study was to determine the toxicity of 30-min exposures of residual chlorine at various temperatures to the freshwater copepods *Cyclops bicuspidatus thomasi* and *Limnocalanus macrurus*. These species were selected for testing because of their importance to the Great Lakes zooplankton community.

Materials and Methods

Laboratory bioassays were performed in accordance with established procedures (Standard Methods 1971; National Academy of Science 1973). All bioassays were conducted in an environmental chamber in which temperature and light were controlled. Cool white fluorescent lighting was supplied on a daily cycle of 16 h light and 8 h dark. Light levels were 40–60 foot candles at the surface of the test aquaria. Experiments were conducted at temperatures of 5, 10, 15, and 20 C.

Water used in the bioassays was obtained from the Linnwood Avenue Water Purification Plant in Milwaukee, prior to any treatment. Lake Michigan water is pumped to the treatment plant from a crib located 1.6 km offshore at a depth of 17 m. The untreated lake water was collected in nontoxic polyolefin barrels and transferred to glass-lined storage tanks in the laboratory. Prior to use, the water was filtered through a 10-μm nitex net to remove zooplankton.

Routine chemical analyses were performed on the Lake Michigan water used in the bioassay system by the Linnwood Avenue Water Purification Plant personnel. Averages of chemical, physical, and biological tests reported for 1974 are presented in Table 1.

TABLE 1. Average chemical characteristics of Lake Michigan water at the Linnwood Avenue Water Purification Plant intake 1974 (data from Milwaukee Water Department).

Water quality test	
total alkalinity ($CaCO_3$)	108.0
chloride	9.7
free ammonia (as N)	0.010
albuminoid ammonia (as N)	0.117
chemical oxygen demand	5.0
dissolved oxygen	12.6
pH	8.21
dissolved solids	160.0
suspended solids	7.0
turbidity (J.T.U.)	3.9
nitrate (as N)	0.287
nitrite (as N)	0.005
phosphate (PO_4)	0.07
coliform bacteria	187/100 ml

All organisms used in the bioassay experiments were collected from Lake Michigan. *Cyclops b. thomasi* was obtained by vertical net tows approximately 5 km offshore; *L. macrurus* by horizontal net tows at depths of 30 m or greater. Most collecting occurred during the summer and early autumn months when *L. macrurus* could not be found in the surface or inshore waters.

Test organisms were maintained in the environmental chamber in 40-liter aquaria, and were acclimated to the temperature of the bioassay for a minimum of 24 h. *Limnocalanus macrurus*, whose upper temperature tolerance limit is about 14 C (Gannon and Beeton 1971), was acclimated to temperatures of 5 and 10 C. The net temperature change between the collection temperature in the Lake and the temperature of the environmental chamber was less than 5 C. *Cyclops b. thomasi*, a eurythermal species, was acclimated to temperatures of 10, 15, and 20 C. Krueger (1973) acclimated *C. b. thomasi* to temperatures ranging from 5 to 25 C, and then abruptly increased the temperature an average of 13.5 C for 30 min with no resulting mortality. In view of Krueger's study, the present acclimation procedures were not considered to be detrimental to *C. b. thomasi*.

The bioassay apparatus consisted of two sets of aquaria. One set of six static aquaria contained lake water to which chlorine was added. The other set of twelve aquaria contained unchlorinated lake water. The unchlorinated aquaria were set up on a continuous flow basis utilizing a modified proportional diluter, based on the design of Mount and Brungs (1967). Lake water was temperature equilibrated and aerated in a head tank prior to passing through the diluter to the flow-through aquaria. The diluter delivered 200 ml of lake water to each of twelve 1200-ml aquaria twice per hour. This flow rate resulted in a 90% replacement time of 7 h.

The vessels in which the test organisms were placed consisted of 12, 235-ml glass jars. Each jar had a 1-cm hole in the side near the bottom that was covered by #10 nylon mesh (153 μm). Lake water flowed into and through the jars while the passage of the test organisms from the jars was restricted. One jar was placed in each flow-through aquarium. Adults and late-stage copepodids of each species were selected microscopically with a dropping pipette. Twenty test organisms were assigned to each of the 12 bioassay jars in a randomized manner to avoid aggregation of easily captured individuals in any one jar.

Six static toxicant aquaria were set up adjacent to the flow-through aquaria, each containing 1 liter of lake water. To five of these aquaria, varying amounts of 5% sodium hypochlorite solution was added to produce a range of residual chlorine concentrations. No chlorine was added to the sixth aquarium which was maintained as a control. Test organisms were placed in jars within the flow-through aquaria approximately 30 min prior to the initiation of the dosing

procedure. At a predetermined time, two sample jars, serving as duplicates, were removed from their respective flow-through aquaria, and all but about 5 ml of the water contained therein was allowed to drain out through the mesh-covered holes. The two jars were then placed in a toxicant aquarium. The chlorine solution filled each jar by flowing in through the screened hole. At the end of the 30-min exposure period, the process was reversed, and the jars were returned to their respective flow-through aquaria.

Any traces of chlorine present in the flow-through aquaria following the return of the jars were gradually removed by means of the continuous flow of lake water through the jars. To determine the efficiency of this dilution process, jars containing 5 ml of a 10 mg/liter chlorine solution were placed in the flow-through aquaria. The chlorine concentration in the aquaria was then monitored. The highest concentration detected was 0.020 mg/liter, measured 20 min after placement in the flow-through aquaria. Within 4 h chlorine was no longer detectable. Based on this information, the dilution process was considered sufficiently effective in reducing chlorine concentrations to negligible levels. This rate of dilution approximates conditions that have been observed in the receiving waters of a power plant effluent.

Immediately following the removal of the jars from the toxicant aquaria, chemical determinations were performed on the toxicant water. Toxicant exposures for the six groups of duplicate samples were staggered at 15-min intervals to allow a 15-min period for determining the total residual chlorine concentration and the pH of the toxicant solutions. Dissolved-oxygen levels in the dilution water were determined occasionally, and were found to vary between 80 and 100% of saturation levels. Total residual chlorine was determined amperometrically (Standard Methods 1971). A three-electrode cell, consisting of stationary platinum working and counter electrodes and a saturated calomel reference electrode (S.C.E.), was connected to a Heath EUA 19-4-2 polarograph. The cell potential was maintained at a constant +0.11 V vs. S.C.E. Mixing in the cell was provided by a teflon-coated magnetic stirring bar driven by a 600-rpm synchronous motor. Cell current was graphically recorded by a strip chart recorder. Titrant was delivered by a Metrohm piston buret. The detection limit of the system was 0.001 mg/liter residual chlorine. The system could not effectively discriminate between free and combined chlorine, so measurements were recorded as total residual chlorine. The accuracy of results obtained with this system was corroborated through the use of iodine standards.

Twenty-four hours after the 30-min chlorine exposure, the bioassay jars were removed from the flow-through aquaria and mortality was assessed. The conditions upon which an individual was judged alive or dead were somewhat arbitrary. Responses to the toxicant were placed in three categories: actually dead, sluggish, and actively swimming. Individuals designated as actually dead showed no response to probing, and appeared contorted and opaque. Swimming individuals displayed levels of activity similar to the control organisms. The intermediate category consisted of individuals that appeared to be partially or completely paralyzed, and that displayed responses to probing ranging from active swimming to weak twitches. In all bioassays except those of *C. b. thomasi* at 20 C, the sluggish individuals were included with the active swimmers to comprise the alive category in the calculation of mortality. For *C. b. thomasi* at 20 C, mortality was determined in two ways. In the first method, as was done in all the other bioassays, the sluggish individuals were included in the alive category. In the second method, the sluggish individuals were included in the actually dead category.

To determine the fate of the sluggish individuals, one bioassay was performed in which six sets of 15 *C. b. thomasi* were exposed to 10 mg/liter chlorine for 30 min, while six sets of 15 were maintained as controls. At 24, 48, and 120 h after the end of the exposure period, two control and two experimental groups were examined to determine numbers of individuals actively swimming, dead, and sluggish. Differences in the numbers of individuals in the three categories with time would tend to indicate whether or not the sluggish individuals ultimately recovered or died.

Statistical analyses of the mortality data were conducted to determine the chlorine concentrations producing 50% mortality following 30-min exposures for each species at each temperature. This median lethal concentration or median tolerance limit is referred to as the TL-50. In each bioassay, percent mortality was determined for each of the 12 samples tested. Percent mortality results for the duplicate samples at each specific concentration were averaged. The average value was plotted on a probit scale vs. the logarithm of the concentration. Results from a minimum of three bioassays for a given species and temperature were plotted on the same graph. The log-probit transformation yields a linear relationship between percent mortality and concentration. The equation of the line passing through the points was determined using the maximum likelihood solution of log-probit analysis developed by Finney (1952). The TL-50 was determined as the concentration corresponding to a mortality rate of 50% on the log-probit regression line. The TL-50 and the slope of the line (regression coefficient) were calculated in each case, as well as the 95% confidence limits for both parameters.

Results

The 30-min TL-50 values for *C. b. thomasi* and *L. macrurus* at various temperatures were calculated from the log-probit regression lines (Fig. 1 A–E). These values are shown, along with the values of the regression slope and the 95% confidence limits of both parameters, in Table 2. The TL-50 value for *C. b. thomasi* at 20 C (5.76 mg/liter) was significantly less than the TL-50 values observed at 10 and 15 C (14.68 and 15.61 mg/liter, respectively). For *L. macrurus*, no variability in the TL-50 value with temperature was

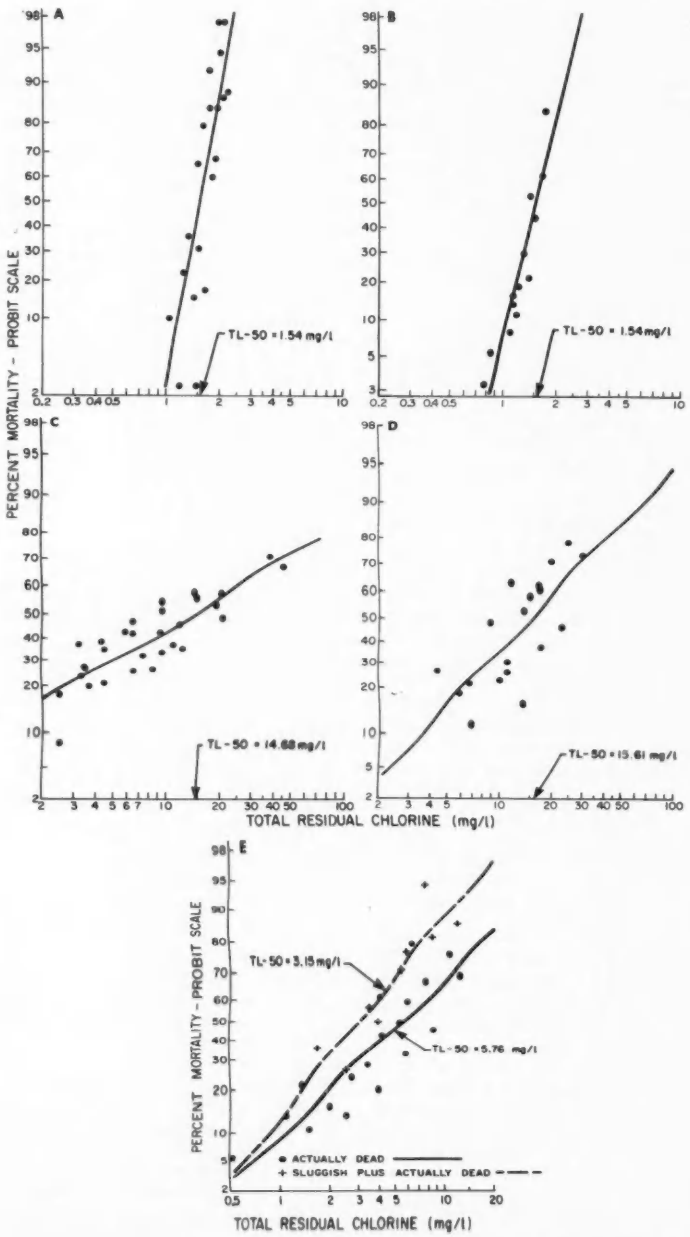

FIG. 1. Dose-mortality plots for a 30-min exposure of residual chlorine to *Limnocalanus macrurus* at A) 5 C and B) 10 C; *Cyclops bicuspidatus thomasi* at C) 10 C, D) 15 C, and E) 20 C. Solid and dashed lines result from mortality calculated by counting sluggish individuals as alive and dead, respectively.

TABLE 2. Statistical results from bioassays in which *L. macrurus* and *C. b. thomasi* were exposed to residual chlorine for 30 min at various temperatures.

Species	Temp (C)	No. organisms tested	TL-50 (mg/liter)	95% Confidence limits for TL-50 (mg/liter)	Regression slope "b"	95% Confidence limits for "b"
C. b. thomasi	10	1411	14.68	12.59–15.29	1.142	0.896– 1.388
"	15	901	15.61	13.96–18.17	2.006	1.386– 2.627
"	20	920	5.76	5.10– 6.99	1.814	1.221– 2.407
"	20[a]	459	3.15	2.29– 3.67	2.357	1.316– 3.397
L. macrurus	5	1208	1.54	1.51– 1.58	7.919	7.097– 9.728
"	10	718	1.54	1.50– 1.58	10.41	6.187–14.63

[a]Mortality calculated by including sluggish individuals in the dead category.

observed. The TL-50 was 1.54 mg/liter at both 5 and 10 C. The TL-50 for *C. b. thomasi* at 20 C was 5.76 mg/liter total residual chlorine, when mortality was calculated by counting the sluggish individuals as alive. However, when sluggish individuals were counted as dead, the resulting TL-50 value was 3.15 mg/liter.

The results of the 5-day bioassay to determine the fate of *C. b. thomasi* rendered sluggish by exposure to chlorine are shown in Fig. 2. While the percent of the individuals of each experimental group actively swimming remained relatively constant at 24, 48, and 120 h, the percent sluggish decreased and the percent dead increased at 120 h. No mortality occurred in the control groups at any time.

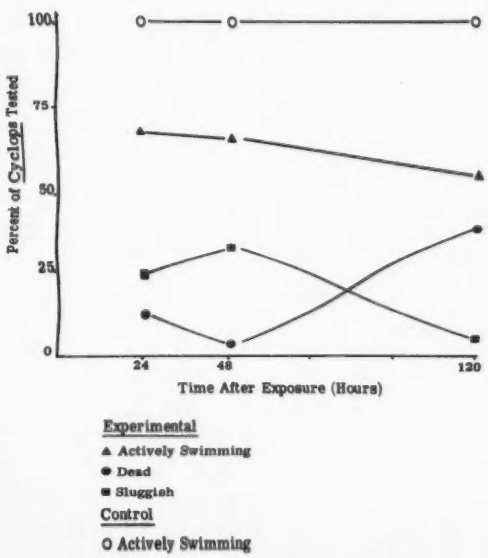

FIG. 2. Results of a 5-day bioassay conducted to determine the fate of *Cyclops* rendered "sluggish" following a 30-min exposure to 10 mg/liter chlorine at 10 C, mortality determined at 24, 48, and 120 h following exposure.

The pH of the toxicant aquaria was observed to increase as the concentration of residual chlorine increased. The pH of the control chambers and the flow-through aquaria ranged from 7.6 to 8.2, over the course of all the bioassays. An incremental increase in the concentration of chlorine of 10 mg/liter generally raised the pH by one-half unit. The pH of the solution with the highest concentration of chlorine tested, 45 mg/liter, was about 9.5. The effect of pH alone on *C. b. thomasi* was determined by performing a bioassay identical to those described above in which the toxicant aquaria contained solutions of chlorine-free lake water to which varying quantities of 1N NaOH were added. This resulted in solutions of pH 8.5, 9.0, and 9.6. One solution of lake water was maintained as a control at a pH of 8.2. No mortality was observed at any of the pH values tested following the 30-min exposure at the elevated pH and the 24-h observation period in unadulterated lake water. This indicates that the mortality observed in the chlorine bioassays was indeed due to the toxicity of chlorine, and not due to pH.

Discussion

The vast majority of *C. bicuspidatus thomasi* and *L. macrurus* were capable of surviving 30-min exposures to residual chlorine at concentrations exceeding the 0.5–1.0 mg/liter range which is characteristic of the effluents of power plants prior to dilution by the receiving water (Draley 1972; Becker and Thatcher 1973). Considerable differences occurred between the two species in regard to the TL-50 values and the regression slopes obtained in the bioassays. *Cyclops b. thomasi* displayed tolerance to concentrations which immediately killed *L. macrurus*. Further,

as evidenced in the differences in regression slope, *C. b. thomasi* was able to withstand a much wider range of concentrations than was *L. macrurus*. Differences in the regression slope may imply differences in the mode of action of the toxicant (Sprague 1969), although this could not be determined in these experiments. Different modes of action appear unlikely, however, considering the similarities in the anatomy and physiology of the two species. The extreme variability in the sensitivity of *C. b. thomasi* to chlorine is evidenced by the observation that at 10 C, 25% of those individuals tested were killed by concentrations less than 5 mg/liter, while 25% were able to survive concentrations of 45 mg/liter. In contrast, about 90% of the *L. macrurus* individuals tested at 10 C were killed over the concentration range 1–3 mg/liter. The high resistance of *C. b. thomasi* to the 30-min exposure to chlorine is in contrast to the 96-h TL-50 of 0.069 mg/liter determined by Beeton et al. (1975). This emphasizes the importance of exposure time when considering the toxicity of chlorine to these organisms.

No experimental attempt was made to determine "safe" levels for 30-min exposures to residual chlorine for the two copepod species studied. However, Sprague (1971) suggests that "safe" levels can be predicted by examining the log-probit regression lines and determining the toxicant concentrations which affect a negligibly small percentage of the individuals tested. Concentrations leading to 5 and 1% mortality, the TL-5 and TL-1, respectively, can be used as estimates of "safe" levels. One advantage of this technique is that the TL-5 and TL-1 statistics are relatively independent of the concentrations used in the experiment. Conventional methods of determining "no effect" concentrations involve exposing test organisms to arbitrary concentrations of toxicant. The resulting highest "no effect" concentration is dependent on the concentrations selected by the experimenter. This problem is avoided by using the TL-5 or TL-1 statistics as estimates of "safe" levels (Sprague 1971).

The TL-5 and TL-1 statistics were calculated from the equations of the regression lines obtained in this study (Table 3). The TL-5 values indicate that *C. b. thomasi* would be protected at 0.5 mg/liter, and *L. macrurus* at 0.9 mg/liter, when exposure periods did not exceed 30 min. Based on the TL-1 values, *C. b. thomasi* would be protected at 0.1 mg/liter and *L. macrurus* at 0.7 mg/liter. It is apparent that these "safe" level estimates exceed the 0.05 mg/liter 30-min exposure that has been recommended (National Academy of Science 1973) and that they roughly coincide with the maximal (0.5–1.0 mg/liter) chlorine range which is typically found in power plant effluents. It is, therefore, concluded that most power plant effluents would have only minor effect on population of *C. b. thomasi* and *L. macrurus*, provided that exposures were limited to 30 min, and that concentrations did not exceed the calculated "safe" levels. At the present time no information is available regarding the long-term effects of a 30-min exposure to residual chlorine. Additional research will be necessary to substantiate the proposed "safe" levels.

Application of laboratory-derived safe levels to the natural environment constitutes additional problems. In the present study, the swimming activity of test organisms was often considerably less than that of the control organisms. These sluggish individuals were apparently suffering from some sublethal effects of exposure to chlorine. Results of the 120-h bioassay to determine the fate of the sluggish individuals indicate that the death of most of these individuals occurs between 2 and 5 days after the exposure to chlorine. This was evidenced by the fact that between 2 and 5 days, the number of sluggish organisms decreased while the number of dead organisms increased. In the natural environment, sluggish individuals would be susceptible to predation, and would face increased competition for food and therefore have a lower survival rate than normal individuals. Laboratory bioassays, in which sluggish individuals are counted as alive, may represent "best case" situations, from which conservative estimates of toxicity are obtained. In light of these conditions, the method of mortality calculation, in which sluggish individuals are counted as dead, may better estimate the effect of chlorine on these species in the natural environment.

Including the sluggish individuals in the dead category resulted in a reduction in the calculated 30-min TL-50 value, and an increase in the probit

TABLE 3. TL-5 and TL-1 statistics used in estimating "safe" levels for 30-min exposures of residual chlorine to *C. b. thomasi* and *L. macrurus*.

Species	Temp (C)	TL-5 (mg/liter)	TL-1 (mg/liter)
C. b. thomasi	10	0.533	0.135
"	15	2.364	1.082
"	20	0.713	0.300
"	20[a]	0.632	0.325
L. macrurus	5	1.073	0.923
"	10	0.956	0.784

[a]Mortality calculated by including sluggish individuals in the dead category.

regression slope for *C. b. thomasi* at 20 C (Fig. 1E). However, the effect on "safe" concentrations was minimal, due to the convergence of the probit regression lines at low mortality levels. Thus, if sluggish individuals had been counted as dead in the bioassays conducted with *L. macrurus* and with *C. b. thomasi* at other temperatures, one might expect somewhat lower TL-50 values, but little change in safe level estimates.

On the other hand, the conditions established in the laboratory may represent a "worst case" situation rather than a "best case" situation, as implied above. In a power plant effluent, exposure time is often considerably less than 30 min, because of the dilution and degradation of chlorine in the receiving waters. The concentration of chlorine in the receiving waters is quickly reduced from the initial effluent levels, because of the chlorine demand of the water and the natural decomposition of chlorine to chloride. Therefore, a laboratory exposure appears as a "worst case" situation from the standpoint of length of exposure. Considering all factors discussed above, the laboratory experiments probably provide a conservative estimate of chlorine toxicity in the field.

Acknowledgments

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Relation Between Egg Size, Growth, and Natural Mortality of Larval Fish

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WARE, D. M. 1975. Relation between egg size, growth, and natural mortality of larval fish. J. Fish. Res. Board Can. 32: 2503–2512.

A set of density-dependent growth and survivorship equations is derived from evidence that the instantaneous death rate in the sea is inversely proportional to particle size. The survivorship equation reproduces several well-known phenomena observed in fish populations. It predicts: 1) that winter and spring spawning species ought to produce larger eggs than summer spawners, 2) that it is advantageous for species that spawn in batches to produce progressively smaller eggs in spring and summer, and 3) that the death rate of a cohort of fish should decrease continuously as the survivors grow and approach the critical size.

The biological basis for the observed variation in the size of pelagic fish eggs and larvae is thought to be due primarily to trophic relations within the pelagic community. It is suggested from what is known of the relative abundance and foraging capabilities of different sized particles, that the survival rates of larval and juvenile fish should increase as they grow and occupy a progressively higher position in the food chain.

WARE, D. M. 1975. Relation between egg size, growth, and natural mortality of larval fish. J. Fish. Res. Board Can. 32: 2503–2512.

En se fondant sur le fait que le taux instantané de mortalité dans la mer est inversement proportionnel à la taille des entités, l'auteur a obtenu une série d'équations où la taille et la survie sont fonction de la densité. L'équation de survie reproduit plusieurs phénomènes bien connus observés chez les populations de poissons. Elle prédit: 1) que les espèces qui fraient en hiver et au printemps devraient produire des œufs plus gros que celles qui fraient en été; 2) qu'il est avantageux pour les espèces qui fraient par périodes répétées de produire des œufs progressivement plus petits au printemps et en été; et 3) que le taux de mortalité d'une cohorte de poissons devrait continuellement décroître à mesure que les survivants grossissent et se rapprochent de la taille critique.

On pense que la cause biologique de la variation observée dans la taille des œufs et des larves des poissons pélagiques provient surtout des relations trophiques qui existent au sein de la communauté pélagique. L'auteur suggère, en se basant sur ce qui est connu de l'abondance relative et de la capacité des entités de tailles différentes de chercher leur nourriture, que les taux de survie des poissons aux stades larvaires et juvéniles devraient s'accroître à mesure que ceux-ci grossissent et occupent une position de plus en plus élevée dans la chaîne alimentaire.

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DESPITE notable differences in the life history and morphology of marine fish that spawn pelagically, it is well known that egg size within a species is remarkably constant for a given geographical area. Though this is true as a generalization, others have noted that egg size tends to decrease in many species throughout the spring and summer. Bagenal (1971) concluded that the observed decline was not correlated with sea temperature or salinity but probably indicates that older, larger fish tend to spawn first. Southward and Demir (1974), however, found that egg diameter of the cornish pilchard (*Sardina pilchardus*) was inversely correlated with sea temperature. Interestingly, they also pointed out that the small summer eggs produced by the Plymouth stock were similar in size to those found in more southern populations.

To explain another well-known seasonal difference in egg size, Cushing (1967) proposed that herring produce eggs that match the type and variability of the production cycle. Comparing autumn, winter, and spring spawners, he found that the latter stocks, which experience greater uncertainty in the development of the

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production cycle, produce large eggs and have a lower fecundity. A similar correlation between egg size, spawning time, and food availability has been reported for plaice (Bagenal 1971).

Several attempts have been made to interpret seasonal variations in egg size from the point of view of larval survival. For instance, it is known that egg and larval sizes are correlated and that larger larvae tend to survive longer without food than those hatched from smaller eggs (Blaxter and Hempel 1963). The adaptive significance of this finding is clear, particularly where the onset of the production cycle is variable (Cushing 1967). Jones (1973) has also suggested, however, that egg size might have evolved so the newly hatched larvae are an appropriate size for feeding on the young growing stages of the plankton. This idea is especially attractive since it implies that the seasonal progression in the size of eggs and larvae, and their subsequent growth and mortality rates reflect regular changes in the structure of the pelagic food chain and hence are ultimately predictable.

It is commonly believed — but has yet to be demonstrated — that the abundance of fish populations is "probably regulated during the larval drift by a combination of density-dependent growth and density-dependent mortality" (Cushing 1974). This paper considers this hypothesis, particularly with respect to the adaptive significance of egg size and the subsequent survival of a cohort of fish during the first year of life. Before I go on to discuss a generalized density-dependent growth and mortality mechanism which appears to account for much of what is known about the early life history of fish, it would be helpful to briefly review some of the evidence concerning egg size and larval mortality.

The Evidence

Variation in Egg Size

To illustrate the size variation in pelagic fish eggs, the egg diameter-frequency for 65 common species is shown in Fig. 1. The bulk of these data were recalculated from Bagenal (1971); the remainder are given by Liem and Scott (1966).

Atlantic halibut (*Hippoglossus hippoglossus*) produce the largest eggs, averaging nearly 3.7 mm in diameter, whereas at the other extreme several species shed eggs as small as 0.7 mm. The mean for all species is 1.2 mm, and the variance is 0.12.

Incubation Time, Temperature, and Egg Diameter

Figure 2a shows the relationship between temperature and incubation time for 14 species of fish that spawn pelagically in the NW Atlantic (Bigelow and Schroeder 1953). In Fig. 2b I have plotted the average size of pelagic eggs discussed by Bigelow and Schroeder (1953) and Liem and Scott (1966), against the probable surface temperature during peak spawning in each case.

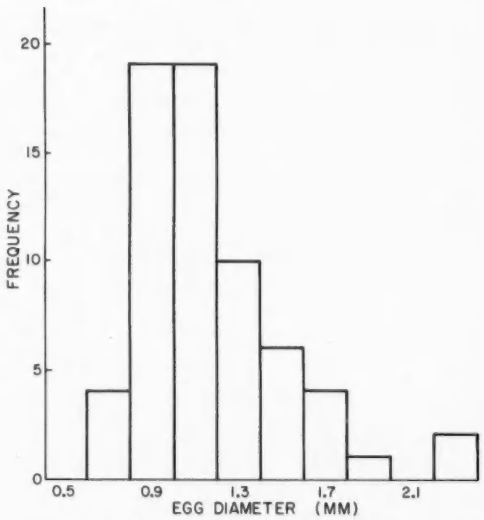

FIG. 1. Pelagic egg size-frequency histogram for 65 common fish species. Four species that produce eggs greater than 2.3 mm in diameter (Bagenal 1971; Liem and Scott 1966) are not shown.

Figure 2c summarizes the data in the two previous figures and indicates that species that spawn at low temperatures, and consequently experience long incubation periods in the field tend to produce large eggs. According to the available information the relation between egg size and incubation time seems to be roughly linear (though with additional data it could become somewhat curvilinear). The circled point, representing Atlantic argentine (*Argentina silus*), is included for interest but was not used in calculating the regression line (Fig. 2c) because its eggs develop at depths greater than 100 fath (Halliday 1974).

Even after allowing for the approximate nature of the data in Fig. 2, there is a clear correlation between egg size and incubation time that remains to be explained. In fact, any hypothesis considering the significance of egg size and the course of larval mortality in the first year of life ought to be able to account for several well-known observations:

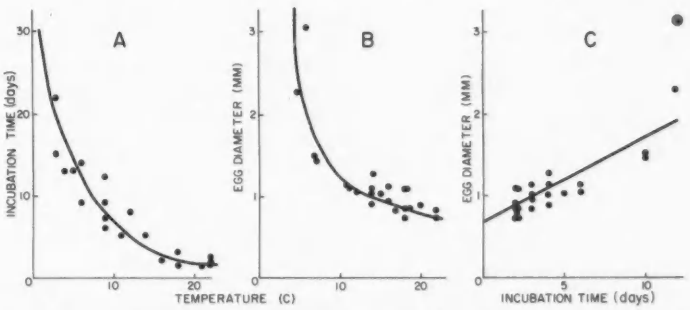

FIG. 2. A, Effect of temperature on incubation time. Bigelow and Schroeder (1953) give two or three incubation times at different temperatures for different species. These are included; B, Relation between average egg diameter and estimated temperature during peak spawning of 23 NW Atlantic fish species; C, Correlation between egg diameter and incubation time (I) in the field, estimated from Fig. 2a according to the probable temperature during peak spawning. $y = 0.101I + 0.67$; $r = 0.87$.

1) The rather small size range in pelagic fish eggs compared to the size variation in females among species; 2) the tendency for large eggs to occur in winter and spring; 3) the general decrease in egg size during the reproductive cycles of spring and summer spawners (Bagenal 1971); 4) the increase in the survival rate of a cohort with time (Marr 1956; Cushing 1974); and 5) a mortality in the order of 3 to 5 logarithmic intervals during the first year of life (Gulland 1965; Bannister et al. 1974; Cushing 1974).

The Hypothesis

Density-Dependent Growth and Mortality

During any small interval of time it is usually assumed that the mass of a fish increases exponentially up to the size at maturity. Also, it follows from the general correlation between body size and doubling time described by Sheldon et al. (1972) that the instantaneous growth rate G tends to decrease as an individual grows according to the familiar power function

$$G = \delta W^{-b}$$

where W = body weight.

This means that

$$\frac{dW}{dt} = \delta W^{1-b}$$

which, when integrated, gives

$$W_t = (\delta b t + W_0^b)^{1/b} \qquad (1)$$

where W_0 = weight of a newly hatched larvae and δ and b are constants.

It is of interest that MacKinnon (1973), using an earlier growth concept developed by Parker and Larkin (1959), derived a similar equation from energetics principles to describe the growth of American plaice. Thus I conclude that the basic form of equation 1 has been verified empirically but, more importantly, can be interpreted in physiological terms.

We have learned from experience that the instantaneous natural mortality rate of fish larvae is not easily estimated. Indeed the variation in abundance of larvae between samples is so great that the 95% confidence interval for each estimate is often about half or double the mean. Our uncertainty in M is further increased as the larvae grow and become less vulnerable to capture. Since net avoidance introduces a systematic overestimate of M, it is important to remember that, even though the investigators whose work is referred to below have taken suitable precautions to minimize sampling errors, their estimates of natural mortality are probably somewhat high. Conversely, G is probably somewhat underestimated.

Beverton and Holt (1959) first pointed out that the growth parameter K in the Von Bertalanffy equation and the annual natural mortality rates M of fish populations are correlated. Figure 3 shows the relation between the average daily growth and mortality rates of haddock (Jones 1973), plaice (Harding and Talbot 1973), and mackerel (Sette 1943) during the first several months of life. Despite the small number of

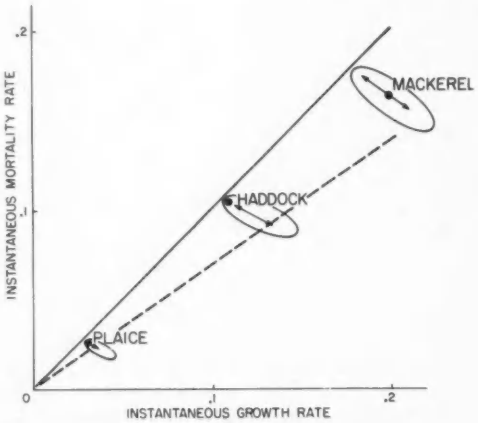

FIG. 3. Relation between the average daily instantaneous growth (G) and mortality (M) rates of plaice, haddock, and mackerel during the first 50–80 days after hatching. The average state of these species probably lies between the solid line where M = G and the dashed line where M = 0.7 G. The ellipses schematically illustrate the probable positions ("fields") of G, M values for a range of good and bad year-classes for each species.

cases involved it is clear that mackerel grow rapidly and suffer a proportionally higher mortality than the two slower growing species. In every case the value of M turned out to be less than G which, of course, it must be if the biomass of a year-class is to increase.

Given the fact that M and G are correlated we can now derive an expression describing the change in abundance of a cohort in time.

Beginning with the usual assumption that

$$\frac{dN}{dt} = -MN$$

But since M = QG, then

$$\frac{dN}{dt} = -Q\delta W^{-b}N$$

which integrates to

$$N_t = \frac{N_0 W_0^{\,Q}}{[\delta bt + W_0^{\,b}]^{Q/b}} \qquad (2)$$

where Q is considered to be a constant and N_0 is the number of newly hatched larvae.

Though this implies that the mortality rate of a species can be estimated from its growth rate, the resulting approximation indicates only the average state of the population. In fact, if average G, M values were plotted in Fig. 3 the points would probably form a straight line and would identify the relative centers of the growth–mortality fields occupied by different species. The relation between M and G is reversed, however, when one considers the respective G, M values for several year-classes within the same population. In this case, as Beverton and Holt (1957) and more recently Cushing (1974) have argued, we would expect to find that when the growth rate was poorer than usual because of an abnormally high larval abundance or decrease in food availability or both, that the larvae would be exposed to the usual sources of mortality for a longer time and their death rate would increase. Hence the G, M values for successive year-classes within a species ought to form a cluster of points lying approximately normal to the G, M regression between species. The simplest way of quantifying this is to assume 1) that δ decreases proportionally as the number of newly hatched larvae increases:

$$\delta = g_0 - g_1 N_0 \qquad (3)$$

and 2) that the mortality rate decreases proportionally whenever δ increases:

$$Q' = g_2 - g_3\delta \qquad (4)$$

where g_0 to g_3 are constants.

Substituting equations 3 and 4 into 1 and 2, respectively, the equations for density-dependent growth and mortality are:

$$W_t = [(g_0 - g_1N_0)bt + W_0^{\,b}]^{1/b} \qquad (1a)$$

and

$$N_t = \frac{N_0 W_0^{\,Q'}}{[(g_0 - g_1N_0)bt + W_0^{\,b}]^{Q'/b}} \qquad (2a)$$

EGG MORTALITY

It is evident from Fig. 1 that the size range of pelagic fish eggs is not great compared to the size variability of the parents. Other observations also indicate that there is even less variation within a species. This suggests that there must be strong selective advantages for conspecific females to produce eggs of a very similar size, rather than having some individuals produce a greater number of smaller eggs or vice versa.

In an evolutionary sense, however, there is no reason (within limits) why a female couldn't produce eggs of any size. The fecundity from a given reproductive mass is inversely proportional to the cube of the egg diameter, D. Also, since the weight of a newly hatched larvae is proportional to the cube of its length or egg volume, the

live weight at hatching is roughly: $W_0 = p\Pi D^3/6$.[1] Hence fecundity and larval size can be calculated for any combination of ovary weight and egg size.

If we now assume that an egg has an equal probability of dying as a larva with the same mass, the number of eggs that survive the incubation period and hatch, N_0, is:

$$N_0 = N_e \text{ EXP } (-IQ\delta W_e^{-b}) \qquad (5)$$

where I = incubation period (d), N_e = initial number of eggs, and W_e = egg weight (milligrams).

To summarize briefly, the foregoing equations propose that growth and mortality in the sea are continuous interdependent processes, and that the instantaneous mortality rate is inversely proportional to particle size regardless of whether it is an egg or larvae. Several obvious implications of this hypothesis can be tested by comparing predicted variations in egg size and mortality rates with empirical data.

TABLE 1. Values used in the calculations.[a]

b	0.232
δ	0.20
Q	0.70
p	0.38
g_0	0.206
g_1	6.63×10^{-10}
g_2	4.2
g_3	17.04

[a]Note that the dimensions for mass = milligrams, length = millimeters, time = days. The growth equation (1, 1a) can be solved when $t > 200$ days by assuming no growth during the "winter." Thus, W at 200 days = weight (MG) of an age 1 fish; W at 400 days = weight (MG) of an age 2 fish, and so forth.

Results

Optimal Egg Size

From an ecological viewpoint it could be argued that variations in egg size and growth rates of fish have evolved to increase the probability of individual survival, on the average, and hence maximize recruitment to the adult stock. Insofar as this is true consider the following:

Assume the bulk of a cohort dies during the first growing season which might last as long as 200 days. Let us also assume that mature females are of similar size and have ovaries of the same weight, but that they have the genetic capability to produce eggs of different sizes. Given a constant ovary weight the selective advantage of various egg sizes can be determined by solving equations 1a and 2a, using the parameter values in Table 1. For example, a 148-g ovary can be divided into 212,366 eggs 1.1 mm in diameter. According to equation 5 if the incubation period is 1 day, 182,377 eggs will hatch. The prolarvae will weigh 0.27 mg (wet weight). At the end of 200 days about 70 fish will have survived and will each weigh about 23 g. Consequently for this particular case, the abundance of the cohort decreased by 3.5 logarithmic intervals, and the average weight of a survivor increased about 4.5 logarithmic intervals.

The relationship between incubation time and optimum egg size, defined as the one leaving the maximum number of survivors after 200 days, is shown in Fig. 4. So far as the best strategy for survival is concerned, it appears that species that experience long, and/or variable incubation periods ought to produce large eggs. By contrast, when the normal incubation period is as short as 1 or 2 days it becomes advantageous to produce small eggs, somewhat greater than 0.5 mm in diameter. This is shown more clearly in Fig. 5 where survival values of different egg sizes are plotted for several incubation periods.[2] In the simulation the selective advantage shifts to small eggs as the incubation period shortens, because the number of eggs that hatch more than offsets the somewhat greater mortality rate subsequently experienced by the larvae due to their small initial size and high abundance. Conversely, the reduction in the average mortality rate of the larvae is not great enough to compensate for the enormous mortality suffered by a small egg during long incubation periods. In principle, therefore, optimal egg size represents a balance between weight and numbers so that enough eggs survive the incubation period, and so individual larvae grow fast enough within the limits of their food

[1] p is a proportionality factor indicating the ratio between the weight of the larvae W_0 and egg.

[2] To simplify the discussion, I acknowledge the established importance of salinity and oxygen availability etc. on egg development and hatching success. Such physiological effects, however, do not appear to change the general argument and therefore are not considered here.

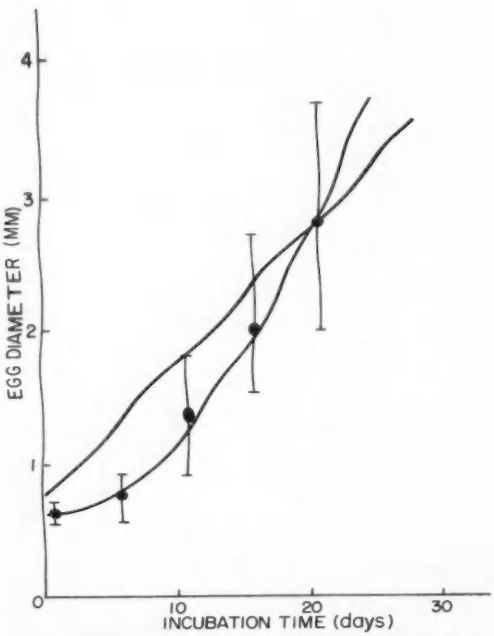

FIG. 4. Comparison of the predicted optimal egg size and the observed size (solid line — Fig. 2c) for various incubation times. The vertical lines indicate that egg sizes within this interval have similar survival values, i.e. ≥ 0.95 times the optimum value.

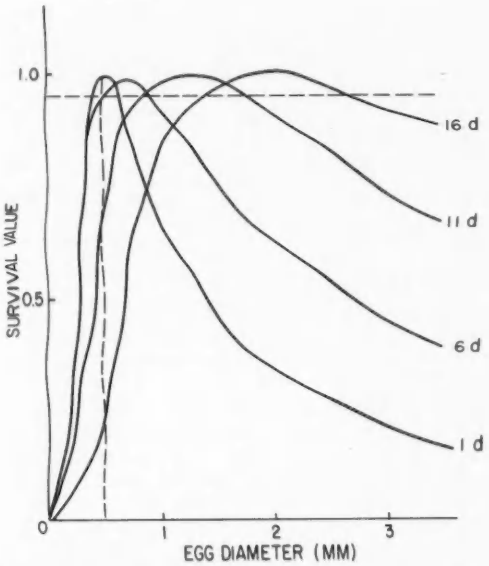

FIG. 5. Relative survival value of various egg sizes over different incubation periods (1–16 days). Survival values were calculated by dividing the number of survivors for each egg size after 200 days by the number of survivors from the optimum sized egg. The latter of course has a survival value = 1.

supply to maximize their chance of survival. According to Bagenal (1971) the commonest fish eggs range from 0.7 to about 4 mm in diameter. In the simulation, eggs of this size had the highest survival values for incubation periods between 2 to 30 days.

Possibly the most complete data on egg loss rates at sea are given by Harding and Talbot (1973) for plaice. Over a 4-yr period the egg mortality rate for the Southern Bight population averaged 7% per day. Using equation 2 the calculated loss rate for a 2-mm diameter egg is 9.5%/day. Sette (1943) reported a somewhat higher loss rate for mackerel eggs ca. 8%/day; by comparison, the calculated mortality rate for a 1-mm egg is about 10%/day. In both cases the calculated estimates probably fall within the confidence limits determined by the field sampling error.

Change in cohort abundance and biomass — To examine some of the consequences of the model more fully, the relative change in abundance and biomass of two cohorts of fish with different growth rates can be compared by considering two cases: a species with a high growth rate such as mackerel,[a] and a slower growing species such as haddock. The results calculated from equations 1 and 2 are plotted in Fig. 6.

Starting with an initial egg mass of 148 g and an egg diameter of 1 mm, the "mackerel" cohort suffered an average mortality rate of 4.6%/day and decreased in number by a factor of $10^{4.4}$ in 200 days. Since the average mass of the survivors increased by a factor of $10^{5.4}$, the biomass of the cohort after 200 days was roughly an order of magnitude higher than the initial egg mass. By contrast, haddock, whose eggs are about 1.5 mm in diameter, decreased by a factor of $10^{3.9}$ (ca. 3.6%/day) over 200 days, whereas the average weight of the survivors increased by $10^{4.1}$. The increase in biomass in this case was therefore roughly double the initial egg mass.

TOWARDS A BETTER MODEL

Density-dependent growth in young fish is well established. Density-dependent mortality, however, is not so well documented though we have incor-

[a]For mackerel $a = 0.154$ and $b = 0.138$.

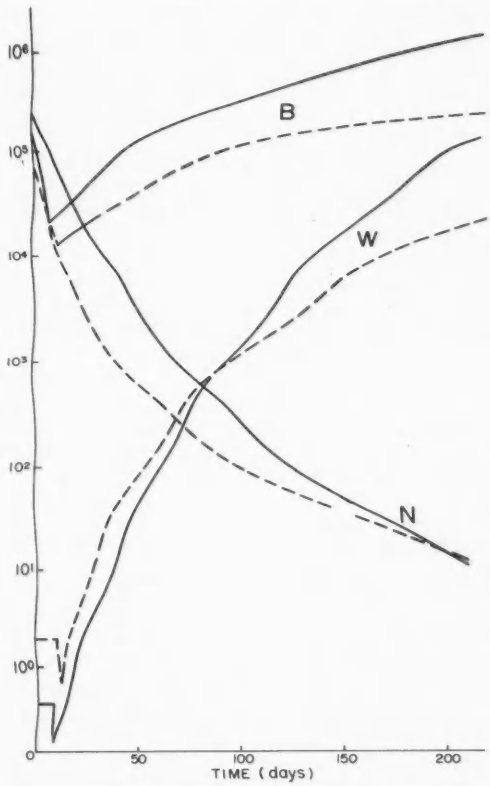

FIG. 6. Simulated changes in average body weight (W, milligrams), abundance (N), and biomass (B, milligrams) of a cohort of mackerel (solid line) and haddock (dashed line) during the first growing season.

porated it into our ecological theories to explain why animal populations tend to remain relatively stable in a virgin state and in the presence of moderate exploitation. The survivorship model (equation 2a) is ecologically stable, since maximum survival at age 1 occurs at intermediate egg densities. Trial simulations also suggest that the model can account for typical fluctuations in year-class strength. For example a one hundredfold change in egg production about the optimum level causes a thirteenfold variation in abundance and an elevenfold variation in biomass at age 1.

Qualitatively then the survivorship equation reproduces much of what is known empirically, but is not completely realistic. A necessary condition for self-regulation is that M must increase at higher densities. So far as growth is concerned, G besides being size-dependent ought to improve whenever the available food:density ratio increases. If we make the usual assumption that the rate of change in population size is proportional to the density, but also assume that M increases whenever G decreases; then we can write a set of simultaneous differential equations where the growth rate is a density-dependent function of body size, and the food supply, whereas the death rate, in turn, is a function of the growth rate and density of the cohort. All else being equal, one would, therefore, expect an inverse relation between body size and abundance. This simple correlation will often be confounded in practice, however, by density-independent variations in food availability.

Discussion

Measurements of the size distribution of particles in the sea show that in any finite volume of water there are many more small particles than large ones. Sheldon et al. (1973) have also pointed out that the amount of particle transfer to deep water (at least in tropical oceans) seems small compared to the production near the surface. From this one might tentatively conclude that predation is a major source of mortality in pelagic communities.

If we argue from another point of view, starting with the fact that G decreases as a particle grows, it is easy to show that large fluctuations in the G:M ratio during the early life history of a year-class would lead to intolerable variations in biomass. Since the normal range in biomass of successive year-classes is often less than a factor of 100, this suggests that the G:M ratio, on average, is only slightly larger than one. Consequently the survival rate should increase constantly as an individual grows and approaches the critical size (where $G = M$).

Though this reasoning implies that growth and mortality rates are linked, an explanation as to why this is so is important because it forms part of the biological basis for variations in recruitment. To explore one possibility in a bit more detail, consider two trophic links in a linear food chain. Let us start with a prey weighing 0.1 units, and a predator weighing 10 units; and its predator, in turn, that weighs 1000 units. If the biomass of the prey in each case is roughly equal to that of its predators (Sheldon et al. 1973; Kerr 1974), and if the average cruising speed (body lengths/day) is inversely proportional to particle size, then the daily food ration (as a percentage of the body weight), and similarly, G and M must decrease as an individual grows. It follows, therefore, that small particles, by moving proportionally faster, search a larger volume of water per

day relative to their body weight than large particles.[4] Since it can be shown that the relative cost of locomotion at the appropriate speed is independent of body size, a small predator, feeding on particles several orders of magnitude smaller than itself, will acquire a proportionally larger ration and will grow at a faster rate than its predator. Here, we again reach the important conclusion that mortality rates in the sea are highest for small particles and continually decrease at each step in the food chain. This argument can be extended to the planktonic community as a whole, as well as to the survivors of a cohort of fish that grow to occupy a progressively higher position in the trophic sequence.

As far as the proximate causes of death are concerned the models developed by Jones (1973) and Cushing (1974, 1975) assume that fluctuations in the timing and amplitude of the production cycle determine the early mortality rate of a cohort. In short they propose that larvae that do not obtain sufficient food die. Whether they succumb to starvation or whether individuals in a nutritionally weakened state are more vulnerable to predation, however, is not known. Although it is clear that starvation is the ultimate determinant of population size and could be an important source of mortality during the first few months of life (Jones 1973), the argument presented above suggests that the normal course of mortality can be predicted qualitatively by assuming that predation is the major cause of death.

The basic distinction between Cushing's model and the one proposed here is that he assumes natural mortality and growth rates are density-dependent functions of age. This is only approximately true, for it is well-known that growth is determined more by the current size of a fish and the food available to it than it is by time alone (Parker and Larkin 1959). Clearly, it would be far more accurate to eventually describe the death process as a size and density-dependent function of the available food supply and predation rate.

In this respect the survival equation described above is obviously oversimplified. Nevertheless, it is interesting to find that by using intermediate values of δ and b most appropriate to a species with the growth characteristics of haddock, and by assuming that an egg is exposed to the same mortality rate as a larvae of similar size that the model is able to generate reasonable egg loss rates in the order of 5–10%/day. When compared with the observed range in egg size, the model also correctly predicted: 1) that eggs between 0.5 and 3.5 mm in diameter ought to have the highest survival values over normal field incubation periods; and 2) that egg size in spring and summer spawners should decrease during the spawning cycle, whereas the opposite trend is expected in autumn spawners. Such variations in egg size are commonly observed (Bagenal 1971; Hempel and Blaxter 1967; Hislop 1975; Southward and Demir 1974). In some cases it is possible as Bagenal suggests that older fish, with larger eggs, might spawn first; however, Hislop (1975) has clearly shown that captive whiting produce large eggs at the beginning of the spawning season. In both whiting (Hislop 1975) and pilchards (Southward and Demir 1974) egg size decreases during the spawning cycle and is negatively correlated with temperature (cf. Fig. 2b).

The adaptive significance of seasonal variations in egg size has been discussed by Cushing (1967), Bagenal (1971), and Jones and Hall (1974). I would add to their comments that whatever the phenomenological basis for the decline in egg size of spring and summer spawners, the temperature trend at that time implies a progressively shorter incubation period, a more advanced production cycle, and higher mortality rates.[5] Presumably the evolutionary pressures of all three circumstances would favor females that produced progressively smaller eggs in spring and summer, and larger eggs in autumn and winter. Also, during the cool, unproductive months of the year large larvae would seem to be better suited for feeding on the overwintering populations of copepods, whereas small larvae with a more restricted feeding range are doubtless better suited for feeding on nauplii and small copepodites that dominate the summer plankton (Jones and Hall 1974). It seems, therefore, that the strategy of reproduction can be interpreted as a compromise between mass and numbers so that 1) the newly hatched larvae are an appropriate size for feeding on the extant zooplankton populations, and 2) so enough individuals survive, on average, within the limits of the available food supply to ensure

[4]There is some evidence for this. The work of Hardy and Bainbridge (1954), for example, indicates that small plankters tend to move proportionally faster for their size (body lengths per second) than large ones. Also, McMahon (1965) showed that the filtering rate (milliliters per hour) in *Daphnia* was roughly proportional to the square of their length.

[5]Equations 5 and 2a take the direct effect of temperature on incubation time into account, but do not consider indirect temperature effects. Higher temperatures, of course, stimulate feeding activity and will tend to increase growth and predation rates throughout the food chain. This tendency will further reinforce the selective advantage for the observed seasonal variations in egg size.

optimum replacement to the adult population. This means that the relation between stock and recruitment is not straightforward, as survival must be determined to some extent by density-independent variations in the food supply and predation pressure in the larval and juvenile stages (Lett et al. 1975; Cushing 1975).

Though the discussion thus far has dealt mainly with the early life history, the survivorship equation also yields reasonable loss rates for a cohort up to the size of maturity. For example, Poulsen's data on the abundance of Baltic cod (cited by Cushing 1974) indicates that the mortality rate of late I to II group fish is in the order of 57% per year. From equation 2 one can calculate for a cohort with the growth characteristics of cod that 2+ yr olds ought to experience an annual mortality rate of about 59%, which decreases steadily to around 34% per year by age 5. The natural mortality rate for Arctic cod greater than 4 yr of age is thought to be about 20–30% per year (Gulland 1965).

Although the agreement between the calculated and observed estimates of prerecruit mortality is not bad considering the approximate nature of the calculations, equations 2 and 2a assume that the difference between G and M decreases with time and approaches, but never quite reaches zero. Of course this is unrealistic since every cohort achieves maximum biomass at some critical size where G = M. What causes M to exceed G so the cohort eventually becomes extinct is important but, surprisingly, is not fully understood. Cushing (1975) has argued that fish eventually become senescent, and that if this process is a physiological property of the individual then the trend in mortality with age after the critical size will not change. It is also possible, however, as Orton originally pointed out (see Gerking 1959; Hislop 1975) that since gonad weight often increases more rapidly in relation to length than body weight, reproduction imposes an increasing metabolic load on older individuals that might ultimately, either directly or indirectly, lead to their death.

Whatever the cause of senescent mortality if it is influenced by physiological condition, then density-dependent variations in the growth of juveniles and adults will not only affect the age of maturity and the mass of reproductive products released by the stock, but might also change the trend in natural mortality in mature age-groups. Since it cannot be universally concluded that density-dependent growth is absent in older fish (e.g. Paloheimo and Kohler 1968; Crabtree and Ware 1975), the importance of this effect on the course of mortality after the critical size becomes of interest.

In summary, it appears from our current knowledge that recruitment in fish populations is regulated largely through variations in growth rates and predation pressure. Although we would eventually like to know whether an individual in poor metabolic condition is more likely to be eaten than it is to die from starvation, the end result is the same. Population size is continuously being adjusted throughout the early life history and beyond the age of recruitment by trophic interactions which, in turn, are governed by the production capacity of the environment. Those populations that cannot sustain adequate growth rates by migrating or finding new food supplies and so forth, to counteract intense predation pressure during the early life history will be unable to replace themselves. Clearly, there are other sources of mortality besides predation, but there is no convincing evidence to suggest that fish stocks are regulated by catastrophic losses during the larval drift. In fact, it seems more reasonable to propose that fish are probably subject to the same basic foraging and energetic constraints that control the overall production and size composition of pelagic communities.

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Fatty Acid Levels in Developing Brook Trout (*Salvelinus fontinalis*) Eggs and Fry

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ATCHISON, G. J. 1975. Fatty acid levels in developing brook trout (*Salvelinus fontinalis*) eggs and fry. J. Fish. Res. Board Can. 32: 2513–2515.

Changes in the fatty acid composition of the yolk and fry of developing brook trout (*Salvelinus fontinalis*) were analyzed. Palmitic (16:0) and docosahexaenoic (22:6) acids were characteristic of the fry and oleic (18:1) and linoleic (18:2) acids were selectively retained in the yolk.

ATCHISON, G. J. 1975. Fatty acid levels in developing brook trout (*Salvelinus fontinalis*) eggs and fry. J. Fish. Res. Board Can. 32: 2513–2515.

L'auteur analyse les changements de composition en acides gras du vitellus et des alevins d'omble de fontaine (*Salvelinus fontinalis*) en voie de développement. Les acides palmitique (16:0) et docosahexaenoïque (22:6) sont caractéristiques de l'alevin, et les acides oléique (18:1) et linoléique (18:2) sont retenus sélectivement par le vitellus.

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ALTHOUGH lipids are of major importance to developing fish both as an energy source (primarily triglycerides) and as a structural component (primarily phospholipids), they have received little research attention. Ando (1962, 1968) and Takama et al. (1969) have studied changes in individual lipid components of developing salmonids but, for the most part, did not distinguish between changes occurring in the yolk and in the embryo proper (fry). Terner et al. (1968) showed that the triglycerides are the predominant lipid components of the yolk of salmonids but that phospholipids play a greater role in the structural components of the fry. Hayes et al. (1973) were the first to directly examine the fatty acid content in the yolk and fry of steelhead (*Salmo gairdneri*) during development.

The purpose of this study was to trace the changes in fatty acid composition of the yolk and fry of developing brook trout (*Salvelinus fontinalis*) and to relate these changes to lipid utilization by the embryo.

Materials and methods — Mature brook trout (*Salvelinus fontinalis*) were obtained from the Grayling Fisheries Research Station, Michigan Department of Natural Resources, and were artificially spawned in the lab. Approximately 400 eggs from two females were incubated in the dark in separate 1000-ml beakers. Water at 11–13 C flowed over the eggs and sac-fry continually. Hatching occurred 40 days after fertilization, and the yolk was completely absorbed by 72 days after fertilization. The sac-fry were not fed, and starvation began soon after complete yolk absorption.

Two samples of 20 eggs or sac-fry were collected every 10 days after the date of fertilization and stored under nitrogen at −15 C until analyzed. Storage time ranged from 6 mo for the early samples to less than 1 mo for the later developmental stages. Sac-fry were separated into a yolk component and a fry component.

The extraction procedure of Bligh and Dyer (1959) was utilized to extract the lipids from the weighed samples. An aliquot was taken for determination of lipid weight and the remainder utilized for fatty acid analysis.

The formation of fatty acid methyl esters was carried out according to the procedures of Metcalf et al. (1966). The sample was saponified with methanolic sodium hydroxide, the fatty acids esterified with BF_3–methanol and the methyl esters extracted with hexane-ethyl ether (1:1) and dried over anhydrous sodium sulfate. The sample was protected from air by a nitrogen atmosphere whenever possible. Methyl esters were stored under nitrogen (storage never exceeded 3 days) in a refrigerator until analyzed.

Fatty acid methyl esters were analyzed with a Hewlett Packard F & M 700 gas chromatograph equipped with a hydrogen flame detector. The columns were ¼ inch by 6 ft stainless steel and packed with 12% diethylene glycol succinate on chromsorb

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TABLE 1. Percentage composition of fatty acid in the total lipid extracted from developing brook trout (*Salvelinus fontinalis*) eggs and sac-fry.

Days after fertilization	Fatty acid composition (%)[a]										
	14:0	16:0	16:1	18:0	18:1	18:2[b]	18:3	20:2	20:3	20:4	22:6
0	2.4	13.8	6.5	5.6	27.4	11.5	4.0	2.8	2.8	6.3	17.0
10	3.0	14.5	7.6	5.6	30.0	13.6	3.9	2.2	2.1	4.6	13.1
20	2.6	13.9	6.7	5.9	27.8	11.8	3.9	3.1	3.0	6.0	15.4
30	2.8	14.6	6.8	5.8	26.6	11.4	4.0	3.0	3.0	6.4	15.6
40 (yolk)	2.4	14.5	5.8	5.6	27.6	11.5	3.9	2.8	3.0	6.4	16.4
40 (fry)	2.3	15.0	6.0	5.9	26.2	11.2	3.6	2.8	3.0	6.4	17.3
50 (yolk)	2.3	13.5	6.4	6.2	28.4	11.9	4.2	2.8	3.0	6.0	15.3
50 (fry)	3.0	17.2	5.6	6.4	24.4	10.2	3.3	2.4	2.4	6.4	18.6
60 (yolk)	3.5	14.1	6.4	6.4	29.2	12.1	4.4	3.0	3.0	5.3	12.8
60 (fry)	2.6	16.3	6.2	6.8	24.2	9.9	3.6	2.6	2.6	6.7	18.5
70	3.2	16.0	6.2	7.4	24.0	10.4	3.3	1.9	2.1	6.2	19.3
80	4.4	19.7	5.6	6.4	20.8	7.5	3.0	2.0	2.2	7.2	21.3

[a]The following fatty acids were present in amounts of less than 1.5%: 8:0, 10:0, 12:0, 14:1, 17:0, 20:1, 22:3, 22:4, and 22:5.

[b]The polyunsaturated fatty acids are ω3, except for 18:2 and 20:4 which are ω6.

P 80-100 mesh. Identification of fatty acid methyl esters was carried out by comparison with standards. Confirmation of the number of double bonds was obtained with thin-layer chromatography on silver nitrate-silica gel G (Morris 1962). The bands were then scraped from the plate, extracted with hexane, and injected into the gas chromatograph.

Results — The average wet weight per egg or sac-fry remained at about 43 mg until after hatching. Subsequently there was a steady increase until the maximum of 60 mg per sac-fry was reached 70 days after fertilization. Lipid weight was stable at about 4.0 mg per egg or sac-fry until between 50 and 60 days, when a 45% lipid loss left an average of 2.2 mg lipid per sac-fry throughout the remainder of the experimental period.

The fatty acid composition of the sac-fry underwent noticeable changes during development (Table 1). The percentage of saturated fatty acids (14:0, 16:0, and 18:0) increased in the embryo after hatching. The only other significant increase in the embryo was in the percentage of docosahexaenoic acid (22:6). Oleic (18:1) and linoleic (18:2) acids showed pronounced decreases in the embryo and a tendency toward concentration in the yolk.

Discussion — The 45% loss in total lipid weight found in this study compared favorably with the results of Ando (1968) and Hayes et al. (1973) for developing rainbow trout (*Salmo gairdneri*). Much of this loss can probably be attributed to the utilization of triglycerides as an energy source with the phospholipids remaining within the fry due to their structural involvement. The timing of this lipid weight loss is significant in that it probably indicates a utilization of triglycerides shortly after hatching. Smith (1952) and Ando (1962) believed that the triglycerides are not utilized until the last stages of yolk utilization. Ando (1968) demonstrated, however, that the triglycerides begin to decrease at a rather steady rate from a time shortly after hatching until complete yolk absorption. Hayes et al. (1973) also found no evidence to support a selective retention of triglycerides in the yolk until just prior to final yolk absorption.

Marked changes occurred in the fatty acid composition of the yolk and fry as development progressed (Table 1). Palmitic acid (16:0) and docosahexaenoic acid (22:6) were characteristic of the fry (i.e. higher precentage in fry than in yolk) and increased with development. Oleic acid (18:1) and linoleic acid (18:2) were selectively retained in the yolk. The remaining fatty acids were fairly stable throughout development. Hayes et al. (1973) also found a tendency for 18:1 to be retained in the yolk and for 16:0 and 22:6 to be preferentially retained in the fry.

Brenner et al. (1963) found that palmitic acid (16:0) was synthesized de novo by the tropical catfish *Pimelodus maculatus*. On a fat-free diet the 16:0 content increased. The largest increase in 16:0 in the present study was during starvation, probably indicating that synthesis was occurring. The increase in the percentage of saturation of total fatty acids was primarily due to this increase in percent 16:0.

Lee et al. (1967) and Hayes et al. (1973) believed that the ω3 fatty acids perform a required role in fish development and growth. They pointed out that 22:6 is the key compound, espe-

cially for phospholipids. Ando (1968) found that 22:6 composed a much higher percentage of the fatty acids in phospholipids (32.2%) than in the triglycerides (16.5%). The 22:6 increases primarily at the expense of 18:3 and possibly other ω3 fatty acids.

This study terminated at the end of the yolk sac stage (with the onset of starvation) as did similar studies reported in the literature. It is not clear how these lipid changes would be altered by the initiation of feeding. This transition phase would seem to be an important area of investigation to demonstrate the significance of changing lipid composition during the late stages of development to the survival of the larval fish.

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Modified Lobster Traps for Catching Crabs and Keeping Lobsters Out

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STASKO, A. B. 1975. Modified lobster traps for catching crabs and keeping lobsters out. J. Fish. Res. Board Can. 32: 2515–2520.

Before redesigning lobster traps for capturing crabs and excluding lobsters, laboratory tests were done to determine the ability of both crabs *Cancer irroratus* and lobsters *Homarus americanus* to pass through simulated trap entrances and escape holes of various sizes and shapes. A long rectangular opening 44.5 mm (1.75 inches) wide allowed most crabs to enter, yet prevented passage of most legal-size lobsters. For use as escape holes in traps, round openings were found to be more effective than square ones to allow escape of lobsters while retaining commercial-size crabs.

Lobster traps were then modified by closing the fishing heads and providing a long rectangular entrance at the top of the trap. Trap entrance widths of 44.5 and 51 mm (1.75 and 2.0 inches) fished crabs equally well; traps with a 38-mm (1.5-inch) entrance, and unmodified lobster traps caught fewer crabs. No lobsters were caught in the modified traps with entrance at the top.

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STASKO, A. B. 1975. Modified lobster traps for catching crabs and keeping lobsters out. J. Fish. Res. Board Can. 32: 2515–2520.

Avant de modifier des casiers à homards de façon à pouvoir capturer des crabes à l'exclusion de homards, nous avons étudié, lors d'essais en laboratoire, l'aptitude des crabes *Cancer irroratus* et des homards *Homarus americanus* à traverser des passages d'entrée et des anneaux de sorties simulés de différentes formes et grandeurs. Une longue ouverture rectangulaire de 44.5 mm (1.75 pouce) de largeur permet à la plupart des crabes d'entrer, tout en empêchant le passage de la plupart des homards de taille légale. Comme ouvertures de sortie des casiers, on constate que des cercles sont plus efficaces que des carrés à permettre aux homards de s'échapper, tout en retenant les crabes de taille commerciale.

Nous avons ensuite modifié les casiers à homards en fermant les cônes d'entrée et en plaçant un long passage d'entrée à la partie supérieure du casier. Des passages de 44.5 et 51 mm (1.75 et 2.0 pouces) capturent tous deux des crabes efficacement; les casiers munis d'une entrée de 38 mm (1.5 pouce) et des casiers à homards non modifiés prennent moins de crabes. Aucun homard n'est capturé dans des casiers modifiés avec entrée sur le dessus.

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IN recent years a small, irregular market has developed for rock crabs (*Cancer irroratus*) (DeWolf et al. 1974). If these crabs are to be fished during the closed season for American lobsters (*Homarus americanus*), steps must be taken to prevent capture of lobsters incidental to rock crab fishing since lobsters support a more valuable fishery than any other single species in Canada. One way to achieve this is by devising a trap that fishes rock crabs and keeps lobsters out.

Several types of traps have been used in experimental fishing for rock crabs in the southern Gulf of St. Lawrence. Younker and McIver (1973) tested five trap types (Japanese queen crab trap, unmodified lobster trap, large Pacific coast Dungeness crab trap, small Dungeness crab trap, and a plastic igloo lobster trap) and found that the ones that caught more crabs also caught more lobsters. Savoie (1970) fished net-covered metal-frame crab traps that also caught lobsters. Modified lobster traps, similar to those described in the present paper but differing in construction of the entrance were used in further experimental crab fishing shortly after the present study was completed in early summer 1974 (M. Albert and J. Branch personal communication; Gallant 1974; D. Younker and L. Murphy personal communication).

The present paper describes laboratory tests on the behavior of crabs and lobsters when presented with simulated entrance and escape holes of different shapes and sizes, and field tests of a modified lobster trap designed to catch crabs selectively.

Laboratory tests — Methods — Three shapes of openings were arbitrarily chosen for testing: a long rectangular opening simulating a trap entrance that would allow the flat-bodied crabs to enter and keep out the round-bodied lobsters; round and square openings simulating escape holes that would allow escape of small crabs as well as lobsters that are small enough to enter through the long rectangular entrance.

A 40 × 40-cm plywood enclosure with 35-cm high sides was set in one of the animal holding tanks, 90 × 90 cm square, filled to 25 cm with sea water at 12 C. The inside of the enclosure was open to room light from above, while the tank area outside the enclosure was shaded with a plywood cover. The simulated trap entrance was a 40-cm long rectangular opening cut out of one wall of the enclosure near floor level leaving a 1.3-cm high sill at the bottom. The vertical width of the opening was adjusted by nailing a board at various distances above the sill — 25.5, 32, 38, 44.5, 51, 57, 63.5, and 70 mm (from 1 to 2.75 inches high in 0.25-inch increments).

To simulate escape holes an opening was cut near the corners in the bottom of each wall of another enclosure and fitted with interchangeable plywood doors. Each such door had one opening cut in it leaving a 1.3-cm sill. The sides of the square openings and diameters of the round ones were 51, 57, 63.5, 70, and 76 mm (2–3 inches in 0.25-inch increments). Tolerance of openings was ±1 mm.

In the tests an animal was placed inside the enclosure and observed until it passed through the opening, or until it had tried and failed. This usually took only a minute or two; the occasional inactive animal was gently prodded to make it active. Two sets of crabs, from 58 to 138 mm shell width in approximately 5-mm increments, were tested once through each opening in a random sequence; four berried females 70, 78, 86, and 97 mm wide were also tested. One set of lobsters from 56 to 103 mm carapace length in approximately 5-mm increments was tested as above with a replicate test being done using the same animals.

Both the crabs and lobsters, originally collected in the north end of Northumberland Strait in the southern Gulf of St. Lawrence, had been kept several weeks in laboratory tanks at St. Andrews, N.B. in unheated sea water 10–15 C. The animals had been fed twice a week and were all vigorous with a hard shell.

TABLE 1. Results of tank experiments to determine what size lobsters and crabs could pass through long rectangular openings. Sizes shown are the largest animals that could pass through.

Width of opening (*mm*)	Lobsters	Crabs	Berried crabs
	Carapace length (*mm*)	Shell width (*mm*)	Shell width (*mm*)
25.5	none	69	none
32	56	79	none
38	62	110	78
44.5	69	121	78
51	83	all	all
57	89	all	all
63.5	92	all	all
70	all	all	all

Results — Rock crabs walked sideways through the three types of openings. When passing through the long rectangular openings the limiting factor was their body depth, i.e. the smallest body dimension of rock crabs. When passing through round and square openings the overall body length (carapace plus protruding abdomen) was the limiting factor. When passing through square openings the crabs, moving sideways, raised the front of the body so that the flattened anterior portion of the carapace fitted in the upper corner of the opening while the posterior part of the body fitted in the diagonally opposite corner.

Behavior of berried crabs differed from the above. They first put the five legs of one side through the opening, then rotated the body putting the anterior end and the other set of legs across the sill, and finally raised the posterior part of the body with egg mass over the sill. Thus, for berried females passing through the long rectangular openings, the limiting dimension was depth of body plus eggs; for passing through round and square openings, the limiting dimension was a compromise between length and width of body.

Lobsters usually walked through the openings head first. Of the 275 observed passes, 92% were head first, 8% were tail first; only rarely did a lobster use its tail to propel itself backwards. In replicate tests the same animal did not walk in a consistent direction. When passing through a vertically narrow rectangular opening lobsters moved upright if there was room, but tilted on one side if the height of the opening was marginal. When passing through round or square openings the lobsters usually put the crusher claw through first, then the other claw, then walked through with claws extended in front. With square openings just large enough to pass through, the lobsters tilted slightly, thus making best use of the diagonal dimension for their vertically oval cross section.

A few crabs and lobsters were pushed through the openings by hand. In all cases the smallest opening that the animal could be pushed through was also the smallest opening that it passed through unaided.

The sizes of crabs and lobsters that passed through the various openings are listed in Tables 1 and 2. Results with the long rectangular openings show that a 38-mm entrance is too small for the larger crabs; a 51-mm entrance would allow all but a few large, exceptionally deep-bodied crabs to enter (Fig. 1). A 51-mm entrance would also let in lobsters up to about 85 mm carapace length.

The possibility of providing an escape hole for lobsters that might enter a crab trap and for crabs smaller than commercial size is indicated

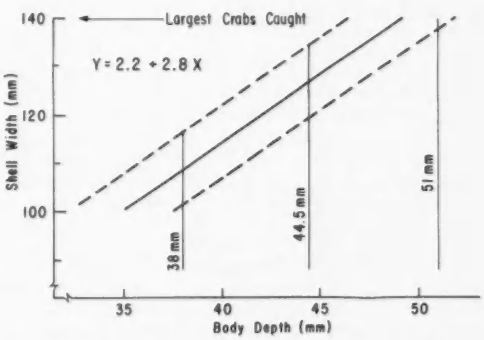

FIG. 1. Regression line (solid) with 95% confidence limits (dotted lines indicate $\pm 2SE = \pm 7.6$ mm) for shell width vs. body depth of commercial-size male crabs 100 mm or more in width. N = 126. Vertical lines indicate the three sizes of trap entrances.

TABLE 2. Results of tank experiments to determine what size lobsters and crabs could pass through square and round openings. Sizes shown are the largest animals that could pass through.

Size of opening	Lobster		Crabs		Berried crabs	
	Carapace length (*mm*)		Shell width (*mm*)		Shell width (*mm*)	
	Round	Square	Round	Square	Round	Square
51	66	72	69	89	none	78
57	72	79	75	100	none	86
63.5	83	89	83	121	78	86
70	92	92	96	all	86	all
76	92	all	110	all	86	all

by results of the tests with round and square openings. The smallest commercial size for rock crabs in the Gulf of St. Lawrence has been 89 mm (3.5 inches) shell width. Crabs over this size will be retained if the escape hole is no larger than 51 mm square or 63.5 mm round. An 85-mm lobster, which is the largest that could enter through the widest of the simulated entrances, could pass through a simulated escape hole 63.5-mm round, but not through a 51-mm square one. Therefore, the round opening should be more selective for retaining commercial size crabs and for allowing lobsters to escape.

The diameter of a round escape hole in crab traps with an entrance up to 51 mm wide should be 63.5 mm with a commercial crab-size limit of 89 mm, at 70 mm with a commercial crab-size limit of 101.5 mm.

Field tests — Methods — Long rectangular entrances were incorporated into lobster traps and field-tested.

The original three-bow lobster traps were about 85 × 50 × 40 cm with a round top. They were covered with laths spaced about 25 mm apart; ends of the trap were covered with netting. The two opposing fishing heads near one end of the trap were 12-cm wooden hoops at the end of horizontal funnels made of netting. Inside the trap another funnel-and-hoop led into a separate compartment or parlour.

Thirty traps were modified as shown in Fig. 2. Modifications introduced to exclude lobsters consisted of lathing over the two fishing heads and placing a long rectangular metal entrance, conical in cross section, at the top of the trap. The entrance, made of 22-gauge galvanized metal sheet, was about 75 cm long, 10 cm deep, and 8 cm wide at the top. Limiting width at the narrow lowest part of the entrance was either 38, 44.5, or 51 mm maintained to a tolerance of ± 1 mm by means of a double-folded stiffener flange and two metal spacers riveted to the flange (Fig. 2b).

The 30 modified traps and 10 unmodified lobster traps (controls) were baited with salted herring and

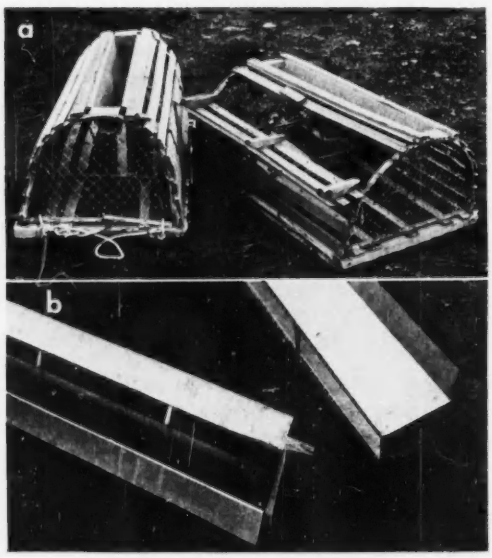

FIG. 2. Modified lobster traps with fishing heads lathed over and a metal entrance on top of each trap (a); detail of entrances (b).

fished on lobster grounds off Neguac, New Brunswick, during the local lobster-fishing season. The traps were set in strings of four — one unmodified lobster trap and three modified ones with entrance funnels 38, 44.5, and 51 mm wide. They were lifted daily, weather permitting, for a total of 8 days, May 22–June 3, 1974. If any one trap in a string of four was damaged, the catch from the whole string was discarded.

Crabs were measured with either a modified metal vernier caliper to which a flat face 30 × 18 mm was welded on each measuring edge, or by a plastic caliper (Watson and Wells 1970). Shell width was the widest dimension across the back, including lateral teeth; body depth was measured from the

TABLE 3. Test fishing with three types of modified lobster traps with entrance at the top, and with unmodified lobster traps, hauled on 8 days from May 22 to June 3. The traps were set in groups of four, each group containing one each of the four types of traps. Totals are number of animals in 51 trap hauls; average is the number of animals per trap haul. Combined numbers are for 204 trap hauls.

Trap type	No. animals					
	Male crabs		Female crabs		Lobsters	
	Avg	(Total)	Avg	(Total)	Avg	(Total)
38-mm entrance	4.8	(246)	3.1	(156)	0	(0)
44.5-mm entrance	8.1	(412)	2.8	(144)	0	(0)
51-mm entrance	8.1	(415)	2.3	(115)	0	(0)
Unmodified entrance	5.7	(291)	2.8	(143)	1.1	(56)
Combined	6.7	(1364)	2.7	(558)	—	(56)

highest point dorsally to the lowest point ventrally. Carapace length of lobsters was measured from the posterior margin of the eye socket to the posterior margin of the carapace in a line parallel to the longitudinal body axis.

Results — Altogether, 51 four-trap strings (204 individual traps) were hauled undamaged after immersion times of 1–3 days (Table 3). Rock crabs were the main catch in all traps, averaging 9.4 crabs per trap haul. Only crabs were caught in the modified traps; the control traps caught crabs as well as lobsters (averaging 1.1 lobsters per trap haul, from 55 to 88 mm carapace length).

The number of female crabs was about equal in each of the four types of trap. However, female rock crabs are of no commercial value since they are smaller than males, reaching a maximum carapace width of about 100 mm in our samples. Best catches of male crabs were made by traps with 44.5- and 51-mm entrances, averaging 8.1 males per trap haul. The unmodified lobster traps (t-test, $P < .01$) and modified traps with 38-mm entrances ($P < .01$) caught fewer male crabs than the other two types.

There is no obvious explanation for the reduced number of male crabs in the unmodified lobster traps; crabs of all sizes were caught in these traps as well as in the modified traps with 51- and 44.5-mm entrances (Fig. 3). By contrast, traps with a 38-mm entrance caught few crabs larger than 110 mm. The factor limiting capture appears to be body depth, the minimum body dimension of crabs (Fig. 1).

The effectiveness of escape holes in allowing lobsters to escape was not tested since no lobsters were caught in the modified traps. Other workers fishing with similar types of traps later in the season and at different locations (see discussion) also had good catches of crabs with only a few lobsters caught during several weeks of fishing.

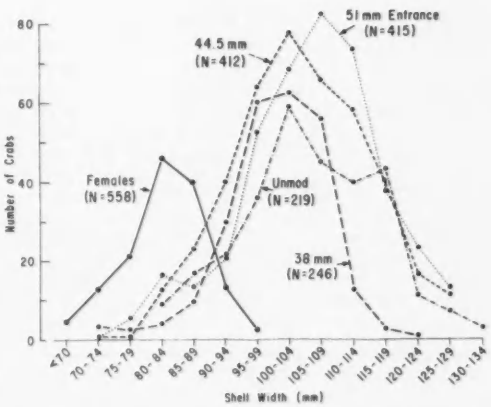

FIG. 3. Size-frequency distribution of rock crabs caught in the four types of traps. Male crabs are shown with broken lines. Since for females the data from the four traps are pooled, the points for females show one quarter of the actual number caught.

Discussion — Both the laboratory and field tests indicate that rock crabs and lobsters readily pass through small openings. The factor that limited passage of an animal that attempted to go through an opening was the size of shell that could be squeezed through. The exception was the unmodified lobster traps that caught fewer crabs yet did not limit the entry of even the largest.

In field tests the modified traps caught more crabs than unmodified lobster traps; no lobsters were caught in the modified traps. In subsequent experimental rock crab fishing off Buctouche, N.B., similar modified traps with entrances made of wood caught only four lobsters in 1861 trap hauls (0.002 lobsters per trap haul) compared to

145 lobsters in 43 trap hauls (3.4 lobsters per trap haul) in unmodified lobster traps (Gallant 1974). Off Georgetown, P.E.I., modified traps with metal entrances caught only 13 lobsters in 1712 trap hauls (0.008 lobsters per trap haul) compared to 1267 lobsters in 808 trap hauls (1.6 lobsters per trap haul) in unmodified lobster traps (D. Younker and L. Murphy personal communication). Preliminary analysis of the Georgetown data indicates that the modified traps fitted with escape holes 44.5 mm square caught fewer small crabs than similar traps without escape holes.

Of the several other types of traps that have been tested by others (Savoie 1970; Younker and McIver 1973) the traps that fished well for rock crabs also fished well for lobsters. For catching lobsters the unmodified wooden lobster trap was better than any of the other types tested.

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Statistical Bias Problem in On-Site Surveys: the Severity of the Problem and its Potential for Solution

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Commercial and recreational fisheries managers often develop catch and effort estimates from information gathered in location or on-site surveys. However, a limited on-site sample cannot produce unbiased estimates of fishing effort or socioeconomic traits of the anglers unless weighting procedures are adopted to account for the varying frequencies of fishing of the fishermen. The corrective procedure involves establishing the relative probability of capturing a fisherman in the sample, then weighting the number of contacts with anglers in each frequency of use category. Unless information on the probability of including particular fishermen and fishing vessels in the sample is available the sample must be drawn with replacement.

Sinclair, W. F., and R. W. Morley. 1975. Statistical bias problem in on-site surveys: the severity of the problem and its potential for solution. J. Fish. Res. Board Can. 32: 2520–2524.

Les responsables de la gestion des pêches commerciales et sportives fondent souvent leurs estimés de prise et d'effort sur des données cueillies au cours de recensements sur le terrain. Cependant, un échantillon limité cueilli de cette façon ne peut que donner des estimés biaisés de l'effort de pêche ou des traits socio-économiques des pêcheurs, à moins d'introduire un procédé

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de pondération qui tiendra compte de la fréquence variable à laquelle le pêcheur pêche. Ce procédé correctif consiste à établir la probabilité relative de capture d'un pêcheur dans l'échantillon, ensuite à pondérer le nombre de contacts avec les pêcheurs dans chaque catégorie de fréquence de pêche. A moins d'avoir à sa disposition des renseignements sur la probabilité d'inclure dans l'échantillon des pêcheurs ou des bateaux de pêche particuliers, l'échantillon doit être prélevé avec remplacement.

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A substantial amount of the information gathered on commercial and recreational fishermen is gathered in location, on-site, or creel surveys. Fishery managers often use location surveys to develop estimates of total fishing effort and total fish catch. In recent years, creel surveys have been used to determine fishermen attitudes, user patterns, or to assess the socioeconomic consequences of particular development projects (Sinclair 1974; Pearse and Laub 1969). They also are used in biologically oriented fisheries studies or in recreationally oriented parks or reservoir studies (Merriam et al. 1972; Jubenville 1971).

The on-site survey method usually involves carrying out controlled user enumeration or interviews in specific locations. On-site surveys owe much of their popularity to the fact that they bring the information gatherer into direct contact with the resource user. This makes it possible to identify easily, and to gather inexpensively some minimal amount of information on resource users who may reside almost anywhere in the world. However, like most other survey methods, location surveys are subject to response bias and interviewer error. More important, they are susceptible to biases which are generated by differences in user participation. It is this type of bias which most often seriously detracts from the accuracy and the reliability of information gathered in location or on-site surveys.

The problem — A major concern in any survey is to ensure that certain segments are neither overrepresented nor underrepresented in what is supposed to be a representative sample of given population. Disproportionate representation can occur because of poor questioning techniques, poor questionnaire design, nonrandom selection of participants, or improper survey design. However, in a location survey disproportionate representation is inherent in the survey technique. User participation bias occurs because certain segments of the population have an excessively high probability of being included in the sample. For example, when interviews are carried out on lakeshores, beaches, in parks, or fishing marinas, people who spend a disproportionate amount of time sunbathing, camping, or fishing have a higher probability of being included in the survey than those who participate in these activities less frequently. Since the more active or more ardent participants will be disproportionately represented, their behavior patterns, attitudes, and opinions will be captured in the survey to the partial or total exclusion of their less devoted counterparts.

Thus, disproportionate representation can occur for two reasons: some persons may stay longer at the site on each visit than others, or some persons may visit the site more often than others. The former is an acknowledged source of error that is recognized in the literature as the "length of stay bias" (Lucas 1963; Lucas and Schweitzer 1965), while the latter is an unrecognized but equally serious source of error referred to here as "frequency of use" bias. Although these two biases are statistically equivalent in that they lead to disproportionate representation, they are two separate problems that can occur simultaneously.

The purpose of this paper is to draw attention to the nature and the extent of frequency of use bias in on-site surveys. Data from a hypothetical shoreline sportfisherman survey will be used to show the potential for this type of bias and how the survey should be adjusted to ensure that the information gathered using this method is a reasonably accurate reflection of the sampled population of sportfishermen.

Frequency of use bias — The information presented in Table 1 describes a hypothetical example of the fishing activity that might occur at a typical fishing location during a given year. From Table 1 it can be seen that of the 250 different anglers that fished during a particular year and at a particular location, 50 different anglers went fishing on 10 different occasions for a total of 500 days of fishing activity. Similarly, 100 different anglers went fishing once during the year for a combined total of 100 days of fishing activity. The data in columns (2) and (3) show, for example, that 20% of the anglers went fishing 10 times during the year, while 40% of the anglers went fishing only once. Hereafter, the four categories in column (3) are referred to as activity groups.

The potential for frequency of use bias in on-site surveys also is demonstrated in the hypothetical example presented in Table 1. For example, assume that the information shown in Table 1 is required to facilitate an improvement in the management of fish stocks. Further, assume that this information is unavailable, and that a limited creel survey is designed to gather data over a short period during the year. Under these circumstances, column (5) shows the probabilities

TABLE 1. Probability of capturing different classifications of fishermen in an on-site survey according to their fishing activity.

	No. anglers	% of all anglers	Days fished per year per angler	Fishing effort each year	Probability of capture
	50	20	10	500	.38
	50	20	8	400	.31
	50	20	6	300	.23
	100	40	1	100	.08
Totals	250	100		1,300	1.00

TABLE 2. Calculations developed from an on-site survey of 20% sample of anglers.

Column	(1) No. anglers in sample	(2) Days fished per year per angler	(3) Estimated no. anglers in each activity category	(4) Estimated percent of anglers in each activity category	(5) Estimated total days fishing effort in each activity category
			$\left(\frac{250}{52} \times \text{column (1)}\right)$		(2) × (3)
	20	10	96	38.4	960
	16	8	77	30.8	616
	12	6	58	23.2	348
	4	1	19	7.6	19
Totals	52		(250)[a]	100.0	1,943

[a]It is assumed that the number of anglers using the site is known or calculated from another source. This is usually the situation when on-site or creel surveys are conducted.

that any angler contacted will be a member of each of the different activity groups. For example, the probability that an angler who is contacted will be a member of the 10 days fished per year activity group is .38; the probability that a contacted angler will be a member of the 1 day fished per year activity group is .08. In other words 20% of the anglers will likely constitute 38% of the interviews, while 40% of the anglers will probably comprise 8% of the interviews. Thus, the hypothetical example shows that there will be a disproportionate representation of different anglers according to the number of occasions they visit the site each year. In reality, the distribution of the sample could be altered (but not without bias) if the population being sampled is small and if the interviews are not repeated (sampled without replacement). However, the distribution would remain the same when the population is large and the survey is conducted over a short period during the year.

Assume, for numerical simplicity, that a sample of 52 anglers (ca. 20% of the population) is obtained in the survey. If the number of anglers (250) who fish at the hypothetical location each year is known, then the basic information that would be gathered in the on-site survey is given in Table 2. The information in column (1) of Table 2 is developed from the probabilities calculated above and shown in Table 1 ($.38 \times 52 = 20$). This information permits the calculation of estimates of the number of days of angling effort that take place at the fishing location. For example, normal interpretation of the information gathered in the survey would suggest, at one extreme, that 96 of the 250 anglers would fish 10 times during the year and at the other extreme that 19 of the 250 anglers would fish only once each year. The number of days fished each year times the estimated number of anglers in each activity group gives the estimated total number of days of fishing effort by activity group as shown in column (5).

A comparison between the given total annual fishing activity (1300 days) provided in Table 1, and the estimated total fishing activity (1943 days) in Table 2, reveals that the location survey would generate an overestimate of angling activity of nearly 50%. The obvious reason for this overcalculation is the overrepresentation of the active anglers and the underrepresentation of the less active anglers in the survey.

The frequency of use bias problem is more serious when the information collected in the on-site survey differs between activity groups. For example, it is generally conceded that the more

TABLE 3. The given and estimated total fish catch per year.

Column	Given				Estimated in on-site survey	
	(1) No. days fished each year per angler	(2) No. fish caught per day per angler	(3) Total fishing effort per year	(4) Total fish catch per year	(5) Estimated total fishing effort per year	(6) Estimated total fish catch per year
				(2) × (3)		(2) × (5)
	10	4.0	500	2,000	960	3,840
	8	2.0	400	800	616	1,232
	6	0.9	300	270	348	313
	1	0.3	100	30	19	6
Totals			1,300	3,100	1,943	5,391

ardent angler has a higher average catch success per day than the less active angler (Sinclair and Sweitzer 1973). Therefore, the angler who goes fishing 10 times a year, on average, can be expected to catch more fish on each fishing occasion than the angler who goes fishing 8 or less times per year. Catch estimates developed from an on-site survey will seriously overestimate the number of fish caught. This problem is illustrated in Table 3 which, using the previous example, shows the given and estimated total fish catch per year. The average number of fish caught per day is combined with the total fishing activity per year (from Table 1) to yield the given fish catch per year. Similarly, the average number of fish caught per day is combined with the estimated total fishing activity per year (from Table 2) to yield the estimated total fish catch per year. A comparison between data presented in column (4) and column (6) shows that the estimated total catch per year exceeds the actual catch per year in the given example by 2291 fish. This is a discrepancy of over 73% between the actual and the estimated catch.

Correction of bias — Frequency of use bias is serious but it need not necessarily nullify the usefulness of on-site surveys. The bias can be eliminated if reliable information on the probabilities of selection in the sample survey can be established (Hansen et al. 1953). The procedure involves establishing the probability of selection for each member of each activity group, then weighting the number of angler interviews in each activity group by the reciprocal of the group's relative probability of selection in the survey.

In a creel survey the relative probability of selection of an angler is dependent on the number of times per year that he goes fishing. For example, an angler who goes fishing 10 days per year is 10 times as likely to be included in a limited creel survey than an angler who goes fishing only once per year. An example of the procedure for correcting on-site bias is provided in Table 4. Column (1) of Table 4 contains the activity group information given in Table 1. With the exception of column (3) (from Table 2), the information in each of the successive columns (2) through (5) is developed from the preceding columns as demonstrated in the table.

The total fishing activity and total fish catch estimates based on the survey information, adjusted for disproportionate distribution, will now coincide with the given figures in Table 3. It should be noted that the number of days fished each year, the number of anglers included in the sample, and the total number of individuals in the target population are required to correct the frequency of use bias problem in a creel survey.

The usefulness of this correction procedure is dependent on two factors. First, the analyst must have an independent estimate of the target population. Second, he must develop reliable information on the probability distribution of the survey interviews over the activity groups. It is important for those using the on-site survey method to be aware that this information is required. If information on the distribution of the sample units over activity groups is not available from an independent source, then the survey must be designed in a manner that permits the development of reliable distribution estimates. This can be accomplished if information on the frequency of participation is gathered in the survey and if care is taken to record the number of repeat contacts in each activity group (sampling with replacement).

It should be stressed that there is no completely satisfactory way of avoiding frequency of use bias without adopting the corrective procedure outlined here. The corrective procedure does not improve the reliability of information gathered in an improperly designed survey. However, the information gathered in a properly designed survey will be an accurate reflection of the entire popula-

TABLE 4. The procedure for correcting the survey sample distribution for user participation bias.

Column	(1) No. days fished each year	(2) Weighting factor[a]	(3) No. anglers included in sample	(4) Survey sample corrected for distribution bias[b]	(5) No. anglers corrected for distribution bias[c]
				(2) × (3)	$\left(\frac{250}{10} \times \text{column (4)}\right)$
	10	.100	20	2.0	50
	8	.125	16	2.0	50
	6	.167	12	2.0	50
	1	1.000	4	4.0	100
Totals			52	10.0	(250)

[a]Weighting factor is calculated by taking the reciprocal of each element in the No. of days fished each year column (i.e. $\frac{1}{\text{10 days fished each year}} = .10$).

[b]Calculated by multiplying every element in column (2) by every element in column (3).

[c]Calculated by multiplying the quotient of the total number of anglers (previously established as 250), divided by the total of the survey sample corrected for distribution bias (10) times every element in column (4).

tion of resource users, and the corrective procedure can be used with reasonable confidence. If information on the probability of users belonging to particular activity groups is obtained from an independent source, then the corrective procedure will only be as reliable as the independent probability information.

Summary — The foregoing discussion has indicated that on-site surveys are prone to severe frequency of use bias and that this is a problem that is frequently encountered in fisheries surveys. The data gathered in location surveys should be used with care and subjected to careful interpretation. If reliable information on the probabilities of anglers and commercial fishermen belonging to particular activity groups is obtained and proper correcting procedures are adopted, then the data can be used with reasonable assurance. The corrective procedure presented here is particularly valuable because it preserves the merit of location surveys and should help improve the usefulness of information gathered in this manner by fisheries managers and researchers.

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Acute Toxicity of Chlorine to Selected Estuarine Species[1]

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ROBERTS, M. H. JR., R. J. DIAZ, M. E. BENDER, AND R. J. HUGGETT. 1975. Acute toxicity of chlorine to selected estuarine species. J. Fish. Res. Board Can. 32: 2525–2528.

Two flowing water systems designed to test the acute toxicity of chlorine in estuarine water are described, one adapted for microscopic species, the other for larger species. Acute toxicity results for several invertebrate and fish species are presented. Molluscan larvae and *Acartia tonsa* were the most sensitive species tested, with 48-h TL50 values less than 0.005 ppm.

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Nous décrivons deux systèmes à eau courante construits dans le but d'étudier la toxicité aiguë du chlore dans les eaux d'estuaire, un système adapté aux espèces microscopiques et l'autre aux espèces plus grandes. Nous présentons les résultats d'essais de toxicité aiguë effectués sur plusieurs espèces d'invertébrés et de poissons. Des organismes étudiés, les larves de mollusques et *Acartia tonsa* sont les plus sensibles, avec des DL50 après 48 h de moins de 0.005 ppm.

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CHLORINE has been widely used in sewage treatment plants because of its strong disinfectant properties and low cost (Brungs 1973). It has been generally asumed that any small residue in the effluent would have little impact on the environment because of the short half-life of chlorine in water. At least in theory, residual chlorine should quickly disappear because of rapid volatilization, conversion to chloride, and dilution.

With increased numbers of larger sewage treatment plants, the validity of presumed low impact has come into question. Large fish kills in the James River, Virginia, in June 1973 and again in 1974 were concluded to have resulted from chlorine released at sewage plants along the river. Ambient chlorine levels as high as 2 ppm were observed in some areas (Virginia State Water Control Board 1974).

Acute toxicity data for free chlorine and chloramines were reviewed by Brungs (1973). Most data were obtained for freshwater species, very little for estuarine species other than salmonids. Brungs (1973) concluded that free chlorine is more toxic than chlorine derivatives such as chloramines.

The chemistry of chlorine in natural waters was briefly reviewed by Brungs (1973). Under ambient conditions in natural waters, chlorine can be expected to form monochloramine by reaction with naturally occurring ammonia. This reaction is accentuated in the presence of high concentrations of amonia in sewage. Other chloramines are a less likely occurrence. However, in sewage, one could also expect formation of various organochloramine and organochlorine compounds. Few studies have been made to identify such compounds (Jolley 1973), and none in estuarine waters.

The objective of the present study was to develop suitable methods for testing several estuarine species and to generate acute toxicity data for these species. Species tested included larvae and juveniles of the oyster (*Crassostrea virginica*), hard clam larvae (*Mercenaria mercenaria*), copepods (*Acartia tonsa*), glass shrimp (*Palaemonetes pugio*), and the fishes, Atlantic silverside (*Menidia menidia*), northern pipefish (*Syngnathus fuscus*), and naked goby (*Gobiosoma bosci*). In designing these tests, it was assumed that chlorination of an effluent is continuous, and therefore concentrations adjacent to outfalls would be essentially constant.

Materials and methods — Test animals with the exception of oyster and clam larvae were obtained by field collection. Field collected animals were held in static aquaria in the laboratory for a minimum of 10

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days prior to testing, with the exception of *Acartia tonsa* which were held 4 days. Oysters and clams were spawned in the lab and used as gastrulae or trochophores in tests.

Palaemonetes pugio, *S. fuscus*, *G. bosci*, and *M. menidia* were fed 2-day-old brine shrimp until 48 h prior to testing, at which time feeding was discontinued. *Acartia tonsa* was fed *Monochrysis* sp. up to the start of testing. Oyster and clam larvae were not fed prior to testing. Juvenile oysters were kept in unfiltered flowing river water without supplemental food. Mortality for all animals used in tests was less than 5% during the 2 days prior to testing, and usually less than 1%.

Two test systems were used for these experiments, both designed to provide as constant a concentration of chlorine as possible. The system used for oyster and clam larvae and copepods (*Acartia tonsa*) was a static, constant addition system. A series of stock solutions was continuously introduced into the 3-liter test aquaria at a rate empirically determined to maintain desired concentrations for 48 h. Aeration was provided as large bubbles in later experiments to prevent establishment of concentration gradients in the aquaria.

All other species were tested in a flow-through system in which both toxicant and diluent were pumped into a mixing chamber and apportioned between two test aquaria at each concentration. Five concentrations and a control were included in each assay.

Stock solutions were prepared by dissolving calcium hypochlorite in deionized water buffered to ca. pH 8. Concentrations of stock solutions remained essentially constant for periods of 24–48 h. The concentrations of stock solutions necessary to produce desired ambient concentrations were determined empirically, since initial losses of chlorine were high. Solutions prepared according to stochiometric calculations resulted in final test concentrations one to two orders of magnitude lower than desired.

The ambient chlorine concentration in each test chamber was assayed at least daily during each experiment. During the first half of the study, analyses were performed by amperometric titration (Fisher amperometric titrater). In several cases, the analysis was performed to distinguish monochloramine from total residual chlorine. In all cases, no monochloramine was detected (detectable limit: 0.02 mg/liter). However, the ambient ammonia levels (0.1–0.3 mg/liter) in the diluent water are such that only combined chlorine should be present, mainly as monochloramine. During the latter half of the study, the prototype of an instrument to measure total residual chlorine electrometrically was available (Marinenko et al. 1975). This instrument was sensitive to as little as 0.002 mg/liter total residual chlorine (readable to 0.001 mg/liter). The total sample size required was only 25–30 ml vs. 200 ml for the amperometric method, a significant advantage.

Temperature, salinity, and dissolved oxygen in the tests were those ambient in the incoming diluent water from the York River. Temperature ranged from 17 C in later experiments to 28 C in experiments conducted in midsummer. During any single experiment, temperature varied by ± 2 C at most. Salinities during the study period ranged from 18.2 to 20.4‰. Dissolved oxygen concentrations were always near saturation.

The TL50 for each species was determined graphically from a graph of percent mortality against log concentration. The mean measured concentration during each test was used in these determinations. The measured response for juvenile oysters was shell deposition rather than mortality. Mean deposition at each test level was expressed as a percentage of deposition by control oysters. The EC 50 for 96 h was then determined in a manner analogous to the TL50 determinations.

Results — The 48- or 96-h TL50 values ranged from 0.28 mg/liter for *S. fuscus* and 0.22 mg/liter for *P. pugio* to less than 0.005 mg/liter for oyster and clam larvae (Table 1).

Several tests were attempted with oyster larvae with varying degrees of success. In an early experiment in which no aeration was used to mix the chlorine and chlorine was added manually at 6–8-h intervals, the apparent 48-h TL50 was 0.11 mg/liter. With constant addition of chlorine stock but no aeration to mix the chlorine, the test results did not permit calculation of a TL50 value, but high mortality was observed at 0.05 mg/liter, the lowest concentration tested. The new instrument for chlorine analysis allowed sampling at surface and bottom and revealed a concentration gradient. Aeration of the test chamber alleviated the gradient. In a test with aeration applied, the TL50 was below the lowest concentration tested, 0.005 mg/liter.

Mercenaria mercenaria larvae were tested in the constant addition system with aeration. The 48-h EC50 based on corrected percent straight hinge larvae was 0.006 mg/liter. (Since only 71% of the control larvae were in the straight hinge stage at the end of the test, the percentage for the control was set at 100% and all other values were corrected by the method of Sprague 1969.) The 48-h TL50 was again less than 0.005 mg/liter, probably on the order of 0.001 mg/liter.

Acartia tonsa were tested in the constant addition system without aeration. The minimum concentration tested was 0.05 mg/liter since that was the minimum detectable level with the amperometric technique in use when this test was performed. The 48-h TL50 was less than 0.05 mg/liter, perhaps on the order of 0.005 mg/liter (based on extrapolation of the data).

The oyster shell deposition test was conducted in the flow-through system. The deep aquaria used in this system are not ideal for this test since water flow is not directed over the oysters. Food availability was also probably less than ideal.

TABLE 1. TL50 (or EC50) values for all species tested in milligrams per liter of total residual chlorine.

Species	Test[a] system	2 h	24 h	48 h	96 h
Invertebrates					
Crassostrea virginica		0.75	0.27	0.11	—
larvae (intermittent Cl_2 addition)	S				
larvae (aerated)	CA	—	—	<0.005	—
juvenile (shell deposition)	FT	—	—	—	0.023[b]
Mercenaria mercenaria					
larvae EC50[c]	CA	—	—	0.006	—
TL50	CA	—	—	0.001[d]	—
Acartia tonsa	CA	—	<0.05	<0.05	—
Palaemonetes pugio	FT	—	0.38	—	0.22
Vertebrates					
Syngnathus fuscus	FT	—	0.28	0.27	0.27
Gobiosoma bosci	FT	0.64	0.08	0.08	0.08
Menidia menidia	FT	—	0.095	0.038	0.037

[a]S: Static system; CA: constant addition system; FT: flow-through system.
[b]Extrapolated; lowest concentration tested 0.04 ppm.
[c]Based on corrected % straight hinge after 48 h.
[d]Extrapolated value since suitable low concentrations could not be tested.

Nevertheless, good shell deposition was observed for the control and some test animals. Since no test animals exhibited a relative shell deposition greater than 30% of the control, an EC50 cannot be derived. If one extrapolates from the data, a 96-h EC50 of 0.023 mg/liter is estimated.

Palaemonetes pugio was tested in the flow-through system. After 24 h, the TL50 was 0.38 mg/liter, after 96 h, 0.22 mg/liter. Inaccuracy in counts at 48 h precluded an estimate of the TL50 for that interval.

Of the three fish species successfully tested, *S. fuscus* was the most tolerant with a 96-h TL50 of 0.28 mg/liter, and *Menidia menidia* was most sensitive with a TL50 of 0.037 mg/liter. *Gobiosoma bosci* was nearly as sensitive as *Menidia* with a 96-h TL50 of 0.08 mg/liter.

Discussion — It is clear from these results that various estuarine species are extremely sensitive to the continuous presence of chlorine in the medium. The most sensitive forms among the invertebrates tested were *Crassostrea virginica* and *Mercenaria mercenaria* larvae and *Acartia tonsa*. The most sensitive fish species was *Menidia menidia*. Both *Acartia* and *Menidia* are important food sources for many commercial species in estuaries.

Juvenile oysters are markedly less sensitive than larval stages. Only a few juvenile oysters actually died during the test, but these mortalities cannot be clearly attributed to chlorine exposure. The TL50 for juvenile oysters would have been greater than the EC50 for shell deposition.

Oyster larvae are markedly more tolerant of chlorine when the exposure is intermittent rather than continuous, with a 48-h TL50 two orders of magnitude higher in the former case than the latter. Even when exposed intermittently, however, the maximum exposure concentration tested was less than ambient concentrations adjacent to some sewage outfalls (Bender et al. 1975).

Acartia tonsa is the dominant copepod in many East coast estuaries, including Virginia tidal rivers, and serves as a major food source for various invertebrates and vertebrates. Continuous exposure to 0.05 mg/liter chlorine permits only 14% survival over 48 h. Dressel (1971) showed that the 96-h TL50 was on the order of 1 mg/liter for a single addition of chlorine at 20 C, and slightly less at 25 C. Dressel's study was addressed to problems of chlorination of power plant cooling water when the biocide is introduced intermittently. In the case of sewage treatment, chlorine addition is more nearly continuous, such that the receiving water adjacent to the outfall reaches a steady state concentration of chlorine (Bender et al. 1975).

The more tolerant species, *Palaemonetes pugio* and *S. fuscus*, feed in part on zooplankton including *Acartia*. Even though themselves relatively tolerant, decreased food supplies could reduce their populations.

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Effect of Bleached Kraft Mill Effluent on the Survival of Starved Juvenile Coho Salmon (*Oncorhynchus kisutch*)

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BROWN, D. A., AND D. J. MCLEAY. 1975. Effect of bleached kraft mill effluent on the survival of starved juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 32: 2528–2530.

Starved coho salmon (*Oncorhynchus kisutch*) fingerlings were exposed to "sublethal" concentrations of neutralized bleached kraft mill effluent (BKME). Time to death was similar in the control group, in fish continuously exposed to BKME concentrations equivalent to 0.05–0.4 of the samples' 96-h LC50 values, and in fish exposed to a treated (foam-stripped) portion of the samples at a volume equivalent to 0.7 LC50 for untreated effluent. Time to death decreased progressively in groups exposed to effluent concentrations higher than 0.4 of the 96-h LC50.

BROWN, D. A., AND D. J. MCLEAY. 1975. The effect of bleached kraft mill effluent on the survival of starved juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 32: 2528–2530.

Des alevins de saumon coho (*Oncorhynchus kisutch*) de la grosseur du doigt, à jeûn, ont été exposés à des concentrations « sublétales » d'eaux usées neutralisées de blanchiment d'une papeterie kraft (BKME). Le temps à mourir est le même chez le groupe témoin, chez les poissons exposés à des concentrations de BKME équivalant à 0.05–0.4 des valeurs de la CL50 après 96 h des échantillons, et chez les poissons exposés à une portion traitée (stripage en mousse) des échantillons à un volume équivalent à 0.7 de la CL50 de l'effluent non traité. Le temps à mourir diminue progressivement chez les groupes exposés à des concentrations d'effluent supérieures à 0.4 de la CL50 après 96h.

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POLLUTANTS discharged to receiving waters at concentrations not immediately lethal may lower the resistance of fish to naturally occurring stressors and thus reduce their chance for survival. Starvation during seasonal lows in food supply is a natural stressor that relates to fish survival. This study examines the effects of sublethal (less than the 96-h LC50) concentrations of neutralized bleached kraft pulpmill effluent (BKME) on

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TABLE 1. Effect of sublethal concentrations of bleached kraft mill effluent on weight at death and time to death.

BKME concentration (*fraction of 96-h LC50*)	Fish wt (*g*)		Time to death (*days*)
	Initial	At Death	
0 (Control)	0.51±0.14[a]	0.38±0.07[a]	47.1±9.8[a]
0.05	0.51±0.15	0.32±0.10	45.4±10.7
0.1	0.56±0.15	0.36±0.06	43.7±9.5
0.2	0.50±0.15	0.33±0.10	43.6±10.4
0.3	0.55±0.20	0.38±0.08	51.5±7.3
0.4	0.49±0.13	0.37±0.05	45.3±13.3
0.5	0.53±0.15	0.44±0.10[b]	33.3±6.2[c]
0.6	0.51±0.11	0.45±0.10[b]	20.8±6.0[d]
0.7	0.57±0.16	0.44±0.10[b]	18.2±4.1[d]
0.7 Treated	0.53±0.17	0.39±0.11	40.6±11.4

[a]Mean ± SD; n = 10.
[b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$. *P* values are based on Student's *t*-test and represent significance of difference between the control sample and the BKME-exposed samples.

survival of fingerling coho salmon (*Oncorhynchus kisutch*) during starvation.

Materials and methods — Juvenile coho salmon, obtained as swim-up fry from the Washington State Department of Fisheries, Samish Hatchery, were held for 3 mo at 12 ± 1 C under natural photoperiod in dechlorinated water (pH 6.5–6.8, conductance 10–25 μmho/cm, EDTA hardness 4–10 mg $CaCO_3$/liter). The fish were fed Oregon Moist Pellets 3 times daily. At the start of the experiment the fish weighed 0.53 ± 0.15 *g*.

Bleached kraft mill effluent samples from a B.C. coastal mill were stored in polyethylene-lined barrels at 2 C until used. The 96-h LC50 value of the effluent was determined each week according to procedures recommended by the American Public Health Association (1971) with 24-h replacement of test solutions: effluent volume to fish ratios were 0.6–0.8 liter/g fish per day, at pH 6.5–6.8, with a minimal aeration rate to maintain the dissolved oxygen content at 9 mg/liter, and temperature 11 ± 1 C. An aliquot sample was detoxified by foam fractionation (Ng et al. 1973, 1974).

Groups of 10 fish were exposed to effluent in 4-liter glass jars at concentrations of 0 (control), 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 of the 96-h LC50, and to detoxified effluent equivalent by volume to 0.7 of the 96-h LC50 value for untreated effluent. The fish were transferred daily to fresh solutions, and were not fed for 24 h prior to transfer from the stock tank or throughout the study. Each fish was weighed initially and at death.

Results and discussion — Time to death was similar in the control group, in fish continuously exposed to BKME concentrations equivalent to 0.05–0.4 of the samples' 96-h LC50 values, and in fish exposed to the detoxified effluent at a volume equivalent to 0.7 LC50 for untreated effluent. Fish died more rapidly at concentrations of 0.5, 0.6, and 0.7 LC50 (Table 1). The larger weights at death of fish exposed to 0.5–0.7 LC50 BKME relative to the control fish (Table 1) may be due to the shorter survival time and incomplete utilization of stored energy reserves in these concentrations.

Similar effects of starvation were observed in brook trout (*Salvelinus fontinalis*) exposed to DDT by Macek (1968). DDT-exposed fish survived an average of 183 days of starvation while untreated fish survived 270 days. These and the present results suggest that concentrations of toxicant which decrease time to death of starved fish may decrease the survival of fish in their natural habitat during seasonal lows in food supply. Such effects could be more pronounced for young fish. Bilton and Robins (1973) showed that 0.5-g sockeye salmon (*Oncorhynchus nerka*) fry survived starvation for 30–50 days which agrees with the data reported here. Previously we found that yearling 10-g coho salmon survived starvation for about 150 days while Macek (1968) records survivals greater than 270 days for starved 80–100-g brook trout.

We conclude that continuous exposure of starved young salmon to bleached kraft pulpmill effluent at concentrations greater than 0.4 of the 96-h LC50 value decreases their chance for survival.

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Crustacean Plankton Communities of the Matamek River System and their Variation with Predation[1]

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POPE, G. F., AND J. C. H. CARTER. 1975. Crustacean plankton communities of the Matamek River System and their variation with predation. J. Fish. Res. Board Can. 32: 2530–2535.

The crustacean plankton communities of lakes with fish planktivores and low densities of *Chaoborus* larvae contained greater numbers of noncyclopoid crustacean predators but lower numbers of the larger herbivores. Lakes with brook trout (*Salvelinus fontinalis*) only and low densities of *Chaoborus* larvae, had plankton communities whose biomasses were dominated by the larger herbivores. The communities of lakes with no fish and high densities of larvae were dominated by the smaller herbivores, although the larger species were present in some. The noncyclopoid, crustacean predators were absent from almost all of these lakes.

POPE, G. F., AND J. C. H. CARTER. 1975. Crustacean plankton communities of the Matamek River System and their variation with predation. J. Fish. Res. Board Can. 32: 2530–2535.

Les communautés de crustacés planctoniques de lacs contenant des poissons planctonophages et de faibles densités de larves de *Chaoborus* contiennent un plus grand nombre de crustacés non-cyclopoïdes prédateurs, mais un nombre moindre de gros herbivores. Les lacs ne contenant que des ombles de fontaine (*Salvelinus fontinalis*) et de faibles densités de larves de *Chaoborus* ont des communautés planctoniques dont la biomasse est dominée par les gros herbivores. Les communautés de lacs dépourvus de poissons et avec hautes densités de larves sont dominés par les petits herbivores, bien que les grosses espèces soient présentes dans certains lacs. Les crustacés non-cyclopoïdes prédateurs sont absents de presque tous ces lacs.

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THE distribution of fish species in the Matamek River System (Fig. 1) was described by Power et al. (1973). Recolonization of the system subsequent to the Wisconsin glaciation is incomplete and many lakes remain fishless. The headwater lakes on the Labrador Plateau that have been reached by fish, contain only brook trout, *Salvelinus fontinalis*, a facultative planktivore. The lakes on the Champlain Plain, between the Plateau and the Gulf of St. Lawrence, contain brook trout and ninespine stickleback, *Pungitius pungitius*, most contain threespine stickleback, *Gasterosteus aculeatus* and rainbow smelt, *Osmerus mordax*, and a few contain Arctic char, *Salvelinus alpinus*, and American eel, *Anguilla rostrata* (Table 1). Of these six species, juvenile smelt and threespine sticklebacks are known to be intensive predators of zooplankton (Pope 1973).

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FIG. 1. The Matamek River System and its location on the Gulf of St. Lawrence (inset). Lakes with fish are colored black; the names of those with brook trout (*Salvelinus fontinalis*) only are underlined.

The distribution and densities of pelagic *Chaoborus* larvae, which are plankton predators, were studied by Pope et al. (1973) and related to the distribution of fish. *Chaoborus americanus* develops relatively dense populations in the fishless lakes on the Labrador Plateau but is absent from other lakes where other species occur at lower densities (Table 1).

Since it is now believed that species composition of crustacean plankton is as much a product of the intensity of predation as of the productivity or physical–chemical nature of a lake (Hrbáček et al. 1961; Brooks and Dodson 1965), an attempt was made to relate the crustacean plankton communities of these lakes to the resident planktivores by comparing the plankton in lakes with: no fish and high densities of *Chaoborus* larvae; brook trout only and low densities of *Chaoborus* larvae; several species of fish including planktivores and low densities of *Chaoborus* larvae.

Methods — The crustacean zooplankton of 26 lakes, 12 with no fish, 6 with brook trout only, and 8 with more than 1 species of fish, were sampled from July 8 to 26 and again from August 28 to September 10, 1970. Three replicate vertical hauls were taken from the bottom at the deepest point in each lake with a conical net (diam 0.5 m) of number 10 mesh (apertures 0.16 mm) and the results averaged. Cladocerans and mature copepods were identified to species. Calanoid copepodids were counted with *Diaptomus minutus* because the vast majority of them were of this species. Also, the developmental stage of *D. minutus* was out of phase among the lakes and to separate the adults and the juveniles of this species would not have facilitated comparison of the communities. The cyclopoid copepodids were classified into a single group while nauplii were ignored. Communities were described in terms of numbers and total volumes per square meter of lake surface. Zooplankton communities are usually described in terms of density but examination of total volume of the species gives a better estimate of energy flow and, therefore, dominance of a species within the community, than of total numbers. Volume also contains a size component and the susceptibility of a plankter to predation is related to size. Quantities were expressed in terms of surface area rather than volume of the lakes because the bulk of the zooplankton are found in the euphotic zone (Pope 1973), which may be a small fraction of the total depth of these lakes. For comparative purposes then, quantities per unit of surface area give a more realistic estimate of density than quantities per unit of volume.

Total volumes were calculated for each species for zooplankton from each lake for each time period from an average length obtained by measuring 10 randomly selected individuals (including juveniles) from that lake at that time. These average lengths were converted to volumes using formulae from table 2.3.1 of I.B.P. Handbook No. 17 (Edmonson and Winberg 1971) and from formulae derived by the authors (Pope 1973).

Comparison of the communities — The distribution and relative quantities of 13 species of crustacean plankton are shown in Fig. 2. The plankton communities of all of the lakes are numerically dominated by *D. minutus*, cyclopoid copepodids, primarily *Cyclops scutifer*, or *Bosmina longirostris*. *Daphnia pulex* is abundant in the August samples from the lakes which contain only brook trout but completely absent from lakes containing additional species of fish, and at low density or absent in fishless lakes. *Diaptomus oregonensis* is most common in the fishless lakes while *Leptodora kindtii* is absent from these lakes and *Epischura lacustris* is present in only one.

When the quantities of the species are expressed as volumes, the larger species such as *Holopedium gibberum*, *C. scutifer*, *Daphnia pulex*, and *E. lacustris* now show a greater significance relative to the smaller species which predominate numerically. *Holopedium gibberum* is particularly important in the July sample from the lakes containing only brook trout.

Tropocyclops prasinus mexicanus, *Diaphano-*

TABLE 1. Selected parameters describing 26 lakes of the Matamek River System.

	Species composition of fish	Maximum density pelagic *Chaoborus* larvae 1970 (m^{-1})	*A* (*ha*)	Maximum depth (*m*)	Conductivity (*μmho/cm 25C*)
Bill	Ar, Ga, Pp, Sf, Om[a]	20	160	29	23.3
Muskrat	Ga, Pp, Om	108	61	28	23.3
Matamek	Ga, Pp, Sf, Sa, Om	15	1320	100	14.0
C21	Ga, Pp, Sf	0	42	7	17.6
Croix	Ga, Pp, Sf, Sa, Om	81	277	73	18.4
C22	Ga, Pp, Sf, Om	46	16	18	16.8
C23	Ga, Pp, Sf, Sa, Om	0	19	12	15.3
Key	Ga, Pp, Sf, Om	15	38	15	15.3
L11	Sf	0	11	7	17.8
Claveau	Sf	0	165	92	13.6
Macrae	Sf	0	20	13	9.3
X	Sf	0	44	31	10.4
L12	Sf	0	12	8	10.2
L13	Sf	20	18	18	11.6
Ross		70	49	49	15.1
Gallienne		75	40	43	14.6
Randin		290	43	67	13.6
L01		530	22	64	12.1
Méchant		80	318	98	14.2
L02		980	10	11	14.4
L03		1200	4	6	10.6
L04		880	18	20	13.2
L05		850	26	23	13.8
L06		1160	28	19	9.1
Tchinicamas		530	94	18	13.8
Tchinicaman		440	429	50	12.3

[a]Ar — *Anguilla rostrata*, Ga — *Gasterosteus aculeatus*, Pp — *Pungitius pungitius*, Sf — *Salvelinus fontinalis*, Sa — *Salvelinus alpinus*, Om — *Osmerus mordax*.

soma leuchtenbergianum, *Polyphemus pediculus*, *Daphnia longiremis*, and *Mesocyclops edax* were also identified from the lakes.

Although there is little difference in the standing crops (by volume) of small herbivores and cyclopoid predators among the three categories of lakes (Table 2), the volumes of large herbivores in lakes with only brook trout are considerably higher than those in lakes with high fish stock and no fish stock. Noncyclopoid predators, *Polyphemus pendiculus*, *Epischura lacustris*, and *Leptodora kindtii* had a very low standing crop in lakes with no fish but high densities of *Chaoborus*.

Discussion — It is obvious then, that there are differences among the plankton communities of the three categories of lakes and these differences are most clearly manifested in the contributions of the large herbivores, and noncyclopoid crustacean predators to the community. The lack of large herbivores (*Daphnia pulex*, *Diaptomus oregonensis*, and *H. gibberum*) in the lakes with fish planktivores is consistent with previous observations (Hrbáček 1962; Brooks and Dodson 1965; Straskraba 1965; Galbraith 1967) that predation by fish is selective on larger individuals of the plankton community and tends to either eliminate them, reduce their numbers, or cause them to mature at a smaller size. In this case, *Daphnia pulex* and *Diaptomus oregonensis* are completely restricted from the lakes. (This is not a geographical anomaly because both species were found in small ponds near the study lakes.) *Holopedium gibberum* was present, but was observed to mature at a smaller size than in the lakes with brook trout only (Pope 1973).

The occurrence of relatively high densities of the predators *E. lacustris* and *L. kindtii* in lakes containing several species of fish is consistent with Dodson's (1970) hypothesis that fish species produce and maintain a suitable niche for a second zooplankton predator by increasing the numbers of zooplankton of a smaller, more desirable size. It appears that in these lakes the predation pressure exerted on *E. lacustris* and *L. kindtii* by fish planktivores is offset by the advantage gained in having a more suitable food source.

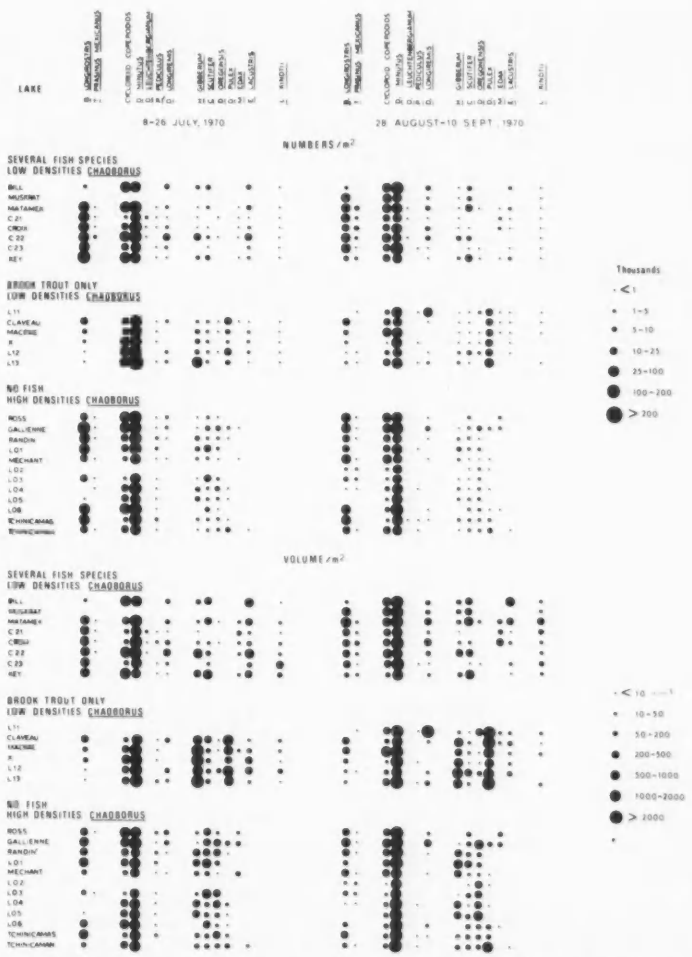

FIG. 2. The relative numbers and volumes of the different species of crustacean plankton in 26 lakes, July 18–23, and August 28–September 10, 1970. The species are listed from the smallest, *Bosmina longirostris* to the largest, *Leptodora kindtii*.

In contrast, the contribution of the large herbivores (*Daphnia pulex*, *H. gibberum*) to lakes containing only brook trout, was great enough that they dominated the community by biomass. This phenomenon in the absence of significant predation has been observed by many (Hrbáček et al. 1961; Straskraba 1965; Hutchinson 1971) and has been expressed in the size–efficiency hypothesis of Brooks and Dodson (1965) which states that when predation is of low intensity, the small planktonic herbivores will be competitively eliminated by the large forms. In this case, however, although the large herbivores have become dominant, the small herbivores have certainly not disappeared and have remained at levels similar to those in all lakes of the system. It appears, then, that competition between the large and small herbivores has not been great in these lakes and that the total standing crops of zooplankton in the lakes with fish planktivores may be suppressed to levels below those in lakes containing only brook trout.

Large crustacean predators occurred in these lakes but at lower densities than in lakes with fish

TABLE 2. Average volume (mm^3/m^2) of various groups of zooplankton in lakes with differing quantities of fish and *Chaoborus* (Fig. 1) for the two sampling periods (July 8–26 and August 28–September 10, 1970).

	All species	Small herbivores[a]	Large herbivores[b]	Cyclopoid predators[c]	Other predators[d]
Heavy fish stock, low densities *Chaoborus*	(8,800)	6,300	200	1,700	630
Light fish stock, low densities *Chaoborus*	(12,300)	5,300	5,700	1,000	260
No fish stock, high densities *Chaoborus*	(7,200)	5,100	990	1,100	15

[a]*Bosmina longirostris, Diaptomus minutus, Diaphanosoma leuchtenbergianum, Daphnia longiremis.*
[b]*Holopedium gibberum, Daphnia pulex, Diaptomus oregonensis.*
[c]*Tropocyclops prasinus mexicanus,* Cyclopoid copepodids, *Cyclops scutifer, Mesocyclops edax.*
[d]*Polyphemus pediculus, Epischura lacustris, Leptodora kindtii.*

planktivores. The standing crop of small herbivores was somewhat smaller, however.

The plankton communities in the fishless lakes shared features with the communities of the other two categories of lakes. *Daphnia pulex* occurred in a few of these lakes but at very low densities. The standing crop of *H. gibberum* was small. *Diaptomus oregonensis* reached its greatest densities in these lakes, but even at these levels its standing crop was very low. The small herbivores dominated the crustacean plankton of these lakes. The large crustacean predators were virtually absent.

The role of *Chaoborus* larvae in shaping this community is unclear. *Chaoborus* may have eliminated the large crustacean predators through competition, although the mechanism by which this would be done is not known. Also, the standing crop of small herbivores upon which both groups of organisms prey is only slightly lower in the fishless lakes than in the other categories of lakes where the crustacean predators occur.

Also, the effect of high densities of *Chaoborus* on the contribution of the large herbivores to the plankton of the fishless lakes cannot be easily explained. *Chaoborus* larvae have been reported to selectively predate larger copepods in the size range of *Diaptomus oregonensis* (Dodson 1970; Sprules 1972) and this selective predation could explain the failure of *D. oregonensis* to become dominant in the community as predicted by the size–efficiency hypothesis of Brooks and Dodson. No such preference by *Chaoborus* for larger cladocerans such as *Daphnia pulex* or *H. gibberum* has been shown, however (Deonier 1943; Roth 1971; Sprules 1972). It appears unlikely, then, that their absence or low biomass in the fishless lakes is attributable to selective predation from any source and that the size–efficiency hypothesis does not hold for these lakes.

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A Small-Boat Otter Trawl for Limnological Studies

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DADSWELL, M. J. 1975. A small-boat otter trawl for limnological studies. J. Fish. Res. Board Can. 32: 2535–2538.

A small otter trawl, 2.5 m wide by 5.5 m long, is described and a method for its use by two operators from a small boat (3–5 m long) powered by an outboard motor is discussed. This small trawl proved efficient in the rapid collection of large numbers of benthic and epibenthic organisms in lakes at depths between 5 and 100 m.

DADSWELL, M. J. 1975. A small-boat otter trawl for limnological studies. J. Fish. Res. Board Can. 32: 2535–2538.

L'auteur décrit un petit chalut à plateaux de 2.5 m de largeur par 5.5 m de longueur et discute d'une méthode d'emploi sur petites embarcations (3–5 m de longueur) propulsées par un moteur hors-bord. Ce petit chalut s'est avéré efficace pour la collection rapide de grands nombres d'organismes benthiques et épibenthiques dans les lacs, à des profondeurs de 5 à 100 m.

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THE use of large otter trawls for commercial and scientific work is common in the oceans of the world and has become highly sophisticated (Carrothers 1968; Clark et al. 1959). Recently their use has spread to the North American Great Lakes, both for commercial fishing (Ferguson and Regier 1963) and scientific surveys (Dryer 1966; Selgeby 1974). These trawls are large, are worked from large boats with inboard engines, and are rigged with powerful winches for trawling.

Small otter trawls, fashioned after the shrimp try-net or the Isaac-Kidd midwater trawl designs, have been employed recently for studies in inland lakes using small boats (Moen 1958; Rupp and DeRoche 1960; De Roche 1969; Van Vliet and Qadri 1970; Reckahn 1970). Most studies have been concerned with fish biology, but recent work has involved zoogeography of fishes and invertebrates (Dadswell 1972, 1974).

The use of small otter trawls in inland lakes is by no means common, though it should be, because it would provide information unobtainable by conventional sampling gear. This paper describes the construction of a small trawl and its operation from small boats using a two-man crew.

Description — The small trawl measures 2.5 m across the footrope and is 5.5 m long (Fig. 1). The headrope is 3.0 m long and buoyed with seven wooden floats. Plastic gillnet floats proved unsuccessful as they were easily damaged. A tickler chain of ¼-inch (6-mm) link, weighing 2 kg, is attached to the footrope (Fig. 1). This causes the footrope to ride slightly above the lake bottom, reducing the accumulation of detritus in the trawl, and causes motile organisms to rise

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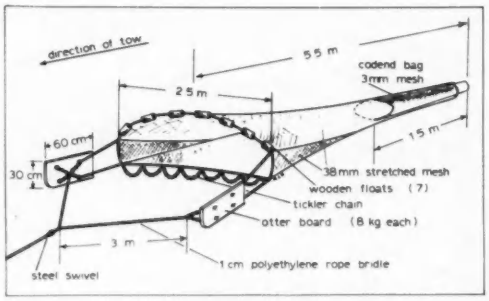

FIG. 1. Scale drawing of small otter trawl.

off the bottom, making capture easier. Alternatively, the chain can be removed from the footrope for deeper penetration of the substrate. A fine mesh window-screening (2.5 m wide × 1 m long) can be attached on the floor of the trawl inside the footrope to direct small heavy organisms, such as molluscs, back to the codend.

Netting is 38-mm polyethylene mesh throughout, lashed with #9 polyethylene twine. The bottom panel is 60 meshes wide at the footrope, tapering in a semiballoon fashion to 35 meshes in the codend. The top panel is 75 meshes wide at the headrope and tapers to 35 meshes in the codend. Side panels are triangular, 17 meshes wide, tapering to a 1-mesh width after 1.5 m (40 meshes).

The headrope and footrope are of 1-cm diameter polyethylene rope. The codend liner is 1.5 mm long, of 3-mm mesh, seine material, constructed of two equal triangular sections sewn together to form a cone open at both ends. The large end of the cone is attached 1.2 m inside the trawl. The liner is closed by tying. Codend liners of different mesh sizes can be interchanged, depending on the organisms being sampled. This liner makes the trawl effective for invertebrates and small fishes. Mesh size of the main trawl body can be varied but should not be less than 30 mm otherwise clogging will result. Minimum liner mesh size could be 1 mm if tows are of short duration.

The otter boards measure 30 cm wide × 60 cm long (Fig. 1). They are attached to the trawl by 1-m sweep lines, which are simply extensions of the headropes and footropes. Attachment is by a double-hitch knot, easily removed after use. The bridle is made of 1-cm diameter polyethylene rope and measures 3 m to a side, thereby forming a rough equilateral triangle with the footrope. A steel swivel is attached at the peak of this triangle. The swivel prevents twisting of the towing line. To facilitate towing we used a single, 1-cm diameter polyethylene rope (breaking strength 2000 kg). This is sufficient to stop the boat without breaking when bottom obstructions are encountered.

This otter trawl can be constructed in about 20 h. The otter boards were purchased from Marinovich Trawl Co., Biloxi, Mississippi. Both this company and Sterling Marine Products, Montclair, New Jersey, have available complete trawls of similar type.

Setting and towing — The advantages of the following trawling method are that operation is simple, portable, and based on human power (more reliable under heavy field use than engines), and that massive samples can be collected rapidly. The disadvantages are that it is hard, backbreaking labor and could be dangerous if care is not exercised; however, approximately 2000 tows were made without mishap during 4 yr.

I used a 4.5-m aluminum boat or a 3.5-m inflatable rubber raft powered by a 9.5-hp outboard motor. Towing was remarkably easy on most bottoms and the trawl offered little resistance to forward movement when the boat was running at 0.5–3 km/h. A larger motor is necessary to obtain higher trawling speeds. The equipment needed for this operation (one boat, one motor, one trawl, one pair otter boards, 300 m of line) can be handled easily by two operators and is portable enough for floatplane or helicopter surveys.

The net is set as illustrated in Fig. 2. Operator 1 controls the motor throughout the operation as well as setting the trawl; operator 2 controls the line and helps with the initial stages of setting. The boat is headed into the wind at moderate speed (½ throttle) and the net played out behind (Fig. 2, position 1). A slow turn is begun, at which time operator 2 releases his otter board over the right side of the boat (inside of the turn) and plays out the bridle (Fig. 2, position 2). As the boat finishes the turn and heads around downwind, operator 1 releases his board and plays out the bridle rope until the bridle junction is held by the outboard motor (Fig. 2, position 3). When both operators are ready, the trawl is released and the line played out at higher speed (¾ throttle) (Fig. 2, position 4). Operator 2 controls the line to ensure even discharge and operator 1 manages the motor with one hand and maintains tension on the line with the other, snubbing the line every 30 m or so of release to maintain tension and to ensure that the trawl remains horizontal. When the desired amount of line has been released, it is tied to the transom as near to the midline of the boat as possible (we usually offset the motor a little to one side), and

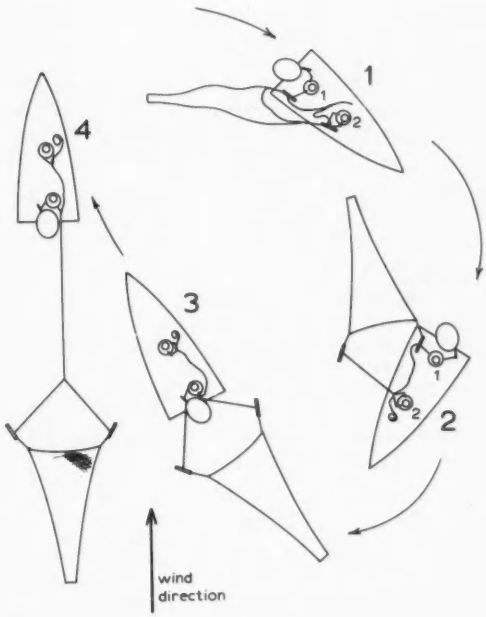

FIG. 2. Method of setting the trawl from a small boat for towing downwind.

towing commences. Trawling downwind is advantageous when setting and hauling since the wind holds the boat off the net making it easier to manage.

Tows generally had a ratio of towline to depth of 3:1. A lower ratio was used for shallower depths and a slightly higher one for greater depths. The buoyancy of a polyethylene line limits the maximum effective trawling depth to about 100 m. Greater depth can be obtained by using steel wire. About ¼ throttle on a 9.5-hp outboard was found to be most effective for towing. Tow duration was 5–7 min. We found that with tows of greater duration, clogging became excessive and recovery of the trawl was more difficult. We seldom towed at depths less than 5 m because bottom obstructions frequently occurred at such depths. The trawl can be towed up almost any bottom incline except a vertical face. We generally attempted to tow on a level bottom although fish capture was more efficient with an upslope tow.

We hauled our net by hand in order to maintain simplicity and portability. A small gasoline engine and winch, as used by Rupp and DeRoche (1960), will lift this trawl easily. A winch arrangement would be useful for long-term studies in a single body of water.

A roller and bracket from a boat trailer was attached to the side of the boat, midship, over which the line was hauled. This greatly reduces resistance and wear, and facilitates the lifting operation. We always used gloves to avoid injury when handling the line at high speeds.

Discussion — The small otter trawl fills a void in the sampling gear available for lake studies. Sampling the deeper portions of lakes has always been difficult, especially for epibenthic, fast-moving organisms or those species with low population densities. Ekman and Peterson grabs are ineffective for collecting motile organisms, whereas the trawl is extremely effective. Up to 5000 *Mysis relicta* have been taken in a 5-min tow. Dredge-nets (Pennak 1953; Schwoerbel 1970) are too light to sample as deep as 100 m; the mouth dimensions are too small to capture effectively fast-moving organisms, and the net tends to clog excessively. Because of its heavy otter boards, large mouth opening, and large mesh size, the trawl has none of these problems. A rigid-mouth dredge-net, large enough to be equally effective, and heavy enough to sample at depth, could not be handled or towed by a small boat. Small-mesh gillnets or traps require excessive sampling effort to capture reasonable sample sizes of small, deepwater fishes. This trawl has captured up to 60 *Cottus ricei* or 30 *Myoxocephalus quadricornis* in one 5-min tow.

The utility of this trawl is clearly seen in its simplicity, handling ease, collecting effectiveness, and portability. During a 4-yr study on the zoogeography and ecology of deepwater organisms in eastern Canada, I obtained a wealth of new information, unobtainable in the same length of time by other means (Dadswell 1972, 1974). The small otter trawl is a useful sampling device and deserves wider use in freshwater studies than it has received in the past.

Acknowledgments — I thank C. Morry, D. Moxley, D. Rivard, J. Kelly, and G. Laver, who served as No. 2 operators during the 4 yr of the study and kept the line from taking me overboard. The late B. Van Vliet built the first otter trawl which I used and it served as a model for later versions. Funds for the study were provided from the National Museum of Natural Sciences through E. L. Bousfield and from the Biogeographic Unit, Carleton University, headed by H. F. Howden. P. J. G. Carrothers critically read the manuscript.

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Edema in Spawning Salmon

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HARGENS, A. R., AND M. PEREZ. 1975. Edema in spawning salmon. J. Fish. Res. Board Can. 32: 2538–2541.

Salmon develop edema during their spawning migration from the sea to fresh water. As measured by the wick technique, fluid pressure in both subcutaneous and peritoneal compartments rises from negative values at sea to positive values in rivers. Concomitantly, blood colloid osmotic pressure falls during the spawning migration. Some of the degenerative changes leading to the death of postspawning salmon probably result from the total-body edema herein described.

HARGENS, A. R., AND M. PEREZ. 1975. Edema in spawning salmon. J. Fish. Res. Board Can. 32: 2538–2541.

Il se forme un œdème chez le saumon au moment de sa remontée de la mer en eau douce. Mesurée par la technique de la mèche, la pression du liquide à la fois dans les compartiments sous-cutané et péritonéal passe de valeurs négatives en mer à des valeurs positives en rivière. En même temps, la pression osmotique des colloïdes du sang baisse lors de la migration de fraie. Certains changements dégénératifs conduisant à la mort du saumon après la fraie résultent probablement d'un œdème de tout le corps tel que décrit ici.

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DURING their spawning migration from a marine to freshwater environment, salmon experience several debilities, any one or a multiplicity of which may be responsible for their hastened death. These degenerative changes include starvation (Patton et al. 1970), liver failure (Trams 1969a), hormonal imbalance (Robertson and Wexler 1959, 1960), loss of neurohomeostasis (Trams 1969b), and the development of massive bacterial and fungal infections.

Osmoregulation and maintenance of body-fluid balance are important parameters in the survival

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of these fishes during their vigorous run from the sea to river spawning grounds. These two functions could also play a central role in the later development of the numerous disorders enumerated above. It was our objective to assess these functions in salmon by monitoring body fluids before and during their spawning migration.

Materials and methods — Four of the five species of Pacific Salmon (*Oncorhynchus*) were examined during two fall spawning migrations. Two fluid compartments and blood were compared in 20 ocean fish and 38 freshwater fish. The marine specimens included eight pink salmon (*Oncorhynchus gorbuscha*), four chum salmon (*O. keta*), two chinook salmon (*O. tschawytscha*), and six coho salmon (*O. kisutch*) which were all caught by commercial purse seines near Namu, British Columbia where salinity averaged 30‰. The freshwater salmons included 22 pinks, 8 chums, 6 chinooks, and 2 cohos, all caught by gillnet in the Atnarko River 40 miles upstream from its mouth at Bella Coola, British Columbia. The salinity from the river's mouth to the spawning grounds was zero. In order to avoid collection variables, all salmon were confined in holding boxes in their marine or freshwater environment for 1 day prior to experimentation.

Body fluids were monitored using 1-mm polyvinylchloride, cottonwick catheters inserted subcutaneously and in the peritoneal cavity of each fish (Scholander et al. 1968; Hargens 1971). Subcutaneous fluid pressure was sampled for changes in peripheral fluid balance, whereas peritoneal fluid pressure represented that in deeper cavities (mesentery and peritoneum). Intramuscular fluid pressure determinations were more prone to bleeding artifacts following wick insertion, and consequently these measurements were discarded. The wick catheter was charged with a slightly heparinized fish Ringer solution and connected to a fine-bored, read-out glass capillary tube which was in turn connected to an adjustable water manometer. The wick method allowed maximal area of fluid contact and rapid attainment of fluid equilibrium. Duplicate subcutaneous and duplicate peritoneal wicks in the same salmon yielded values ±0.3 cm H_2O and ±0.1 cm H_2O, respectively.

Samples for blood analyses were withdrawn from the posterior end of the caudal vein. Colloid osmotic pressure was measured using a modified, stretched dialyzing membrane technique (Hargens and Scholander 1969) requiring a 0.2-ml blood sample. The contribution of the protein-anticoagulant, heparin, to this determination was subtracted. Duplicate hematocrits were taken for each fish blood sample to verify sample quality and as a further check on blood concentration. The condition of each fish caught in the river (pre- and postspawning) was noted.

Results — All four species of Pacific salmon sustained increased body-fluid pressure upon migration from sea to river water (Fig. 1). Three of them (pink, chinook, and coho) developed edema in both their subcutaneous and peritoneal compartments. The chum salmon underwent some rise in body-fluid pressure, but not as great as the others. This was probably a consequence of sampling the chum salmon early in their spawning run and their brief exposure to a freshwater environment as evidenced by their healthy, prespawning condition. As is common in most animals under natural conditions (Scholander et al. 1968; Hargens 1971), all ocean salmon possessed negative body-fluid pressure. The positive, edematous body-fluid pressure found in river salmon indicates fluid entrapment by impermeable cutaneous layers around the body compartment. It was also noted that most postspawning fish that had lost spatial equilibrium and were on the brink of death had body-fluid pressures near ambient. Their numerous and extensive open wounds probably allow fluid leakage from the body compartment resulting in zero fluid pressure readings.

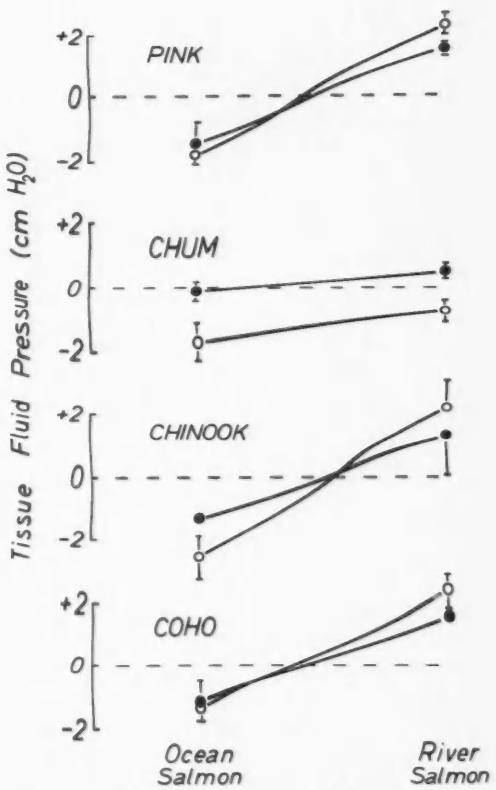

FIG. 1. Development of edema or near edema in four species of Pacific salmon: pink (*Oncorhynchus gorbuscha*), chum (*O. keta*), chinook (*O. tschawytscha*), and coho (*O. kisutch*) upon migration from ocean to river water. ●, peritoneal fluid pressure; ○, subcutaneous fluid pressure. Values are means ± SD.

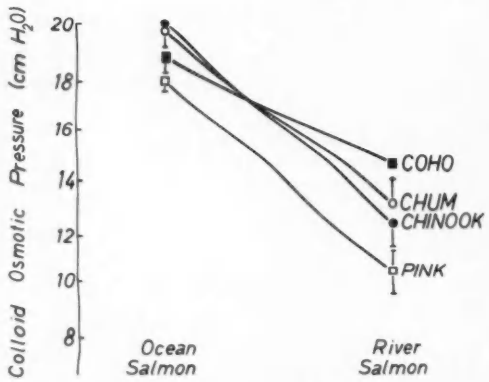

FIG. 2. Fall of blood colloid osmotic pressure in four species of Pacific salmon upon migration to spawning grounds: □, pink; ○, chum; ●, chinook; and ■, coho. Values are means ± SD.

Concomitant with the rise in body-fluid pressure, all fishes had significantly lower blood colloid osmotic pressures upon migration from ocean to fresh water (Fig. 2). This fall in plasma protein concentration was probably due to a number of factors: a partial freshwater flooding of the fishes (supported by our observations that total salt concentration and hematocrit also decrease), starvation of these fishes during their spawning migration (Patton et al. 1970), and conversion of plasma proteins into reproductive endproducts (Phillips 1969).

Discussion — Robertson et al. (1961) found a marked decrease in blood total-protein concentration in chinook salmon during their spawning migration. However, the extensive body edema herein ascribed to river salmon has not been previously reported. The onset of this nonadaptive, pathogenic condition is probably due in part to the lower blood colloid osmotic pressure in these river fishes. In addition, circulatory aberrations could also promote the edematous condition. We found a significantly lower Ca^{+2} concentration in river fishes, and this in itself produces increased capillary filtration (Nicolaysen 1971). Furthermore, lower Ca^{+2} concentration is associated with decreased fluid return flow via the lymphatic system (Mislin and Schipp 1967). The high capillary permeability in teleost fishes as a whole (Hargens et al. 1974) would aggrevate any edema caused by a fall in blood colloid osmotic pressure.

It is possible that some of the degenerative changes to which spawning salmon are so prone are due solely to changes in body-fluid balance upon their migration into fresh water. Epidermal lesions (Brinkmann 1962; Shelton and Wilson 1973), lymphocystis and ulcers (Woodcock 1904; Johnstone 1905) are all common to marine fishes caught in periodic sea pockets of low salinity. One of us has found that marine fishes develop edema immediately upon exposure to freshwater (Hargens unpublished data). Finally, it is noteworthy that the circulatory degeneration developed in steelhead trout (*Salmo gairdneri*) during their spawning migration into fresh water is reversible upon their reentry into the sea (Van Citters and Watson 1968).

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First Occurrence of the Brook Silverside, *Labidesthes sicculus*, in a Tributary of Lake Superior

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BERG, R. E., P. A. DOEPKE, AND P. R. HANNUKSELA. 1975. First occurrence of the brook silverside, *Labidesthes sicculus*, in a tributary of Lake Superior. J. Fish. Res. Board Can. 32: 2541–2542.

On June 4, 1974, three brook silversides, *Labidesthes sicculus*, were collected by electrofishing near the mouth of the Dead River, a tributary of Lake Superior at Marquette, Michigan. This is the first record of the species in the Lake Superior watershed and it may be that the species has only recently established itself in the area.

BERG, R. E., P. A. DOEPKE, AND P. R. HANNUKSELA. 1975. First occurrence of the brook silverside, *Labidesthes sicculus*, in a tributary of Lake Superior. J. Fish. Res. Board Can. 32: 2541–2542.

Le 4 juin 1974, les auteurs capturaient trois crayons d'argent, *Labidesthes sicculus*, au moyen de la pêche à l'électricité près de l'embouchure de la Dead River, tributaire du lac Supérieur à Marquette (Michigan). C'est la première fois que la présence de cette espèce est signalée dans le bassin versant du lac Supérieur et il est possible qu'elle ne se soit établie dans la région que récemment.

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SHORTLY after nightfall on June 4, 1974, three brook silversides, *Labidesthes sicculus*, were collected with a boat-mounted 230-V A.C. boom-shocking unit during a monthly survey of fish populations in the Dead River. The Dead River is a tributary of Lake Superior at Presque Isle Harbor, Marquette, Michigan. One fish was collected in Lake Superior at the mouth of the river, while two others came from an area about 100 m upstream from the mouth. One specimen was released; the other two, both gravid females 81 and 72 mm in total length, were preserved in 10% formalin.

Neither Hubbs and Lagler (1964) nor Christie (1974) reported this species in the Lake Superior drainage. Cahn (1927) and Greene (1935) placed the species in the headwaters of the upper Fox River and in Shawano Lake, Lake Michigan watershed and also in Chain-O-Lakes, northwestern Washburn county, Mississippi River watershed, all in Wisconsin. Scott and Crossman (1973) reported

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its occurrence in lakes Ontario, Erie, and St. Clair, and in Georgian Bay of Lake Huron. The brook silverside has not previously been found in the Lake Superior watershed according to Dr R. M. Bailey, University of Michigan (personal communication).

The specimens were captured in a slow moving portion of the Dead River, off the main channel. The water temperature was 18 C on the surface. The water depth ranged from 0.5 to 1.5 m. Rooted aquatic vegetation is abundant in this section of the river.

How the brook silverside came into the watershed may never be known. The lack of prior records for this species in Lake Superior suggests either that it is indigenous but very rare or that it has recently appeared. Either way the population is quite small. R. M. Bailey (personal communication) suspects the species was introduced into the area, but migration through the locks at Sault Ste. Marie remains a possibility.

One of two preserved specimens of *L. sicculus* was sent to the Museum of Zoology, the University of Michigan for verification. The catalogue number is UMMZ 196800 (one specimen). The other remains at Northern Michigan University, catalogue designation NMZ 70.

Acknowledgments — We extend appreciation to the Marquette Fisheries Research Station, Michigan Department of Natural Resources for use of the electrofishing unit for this study.

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Seaward Movement of Juvenile Fishes, Including Lunar Periodicity in the Movement of Coho Salmon (*Oncorhynchus kisutch*) Fry

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MASON, J. C. 1975. Seaward movement of juvenile fishes, including lunar periodicity in the movement of coho salmon (*Oncorhynchus kisutch*) fry. J. Fish. Res. Board Can. 32: 2542–2547.

Following emergence, more than 51,000 coho salmon (*Oncorhynchus kisutch*) fry moved seaward from Lymn Creek. Peaks in movement occurred coincident with the new moon and progressively declined through four lunar cycles although 75% of the outmovement took place between early April and the middle of June. Coho smolt migration occurred simultaneously, the run peaking in late May during a period of maximum low tides between sunrise and sunset when the moon was full. Sticklebacks (*Gasterosteus*) moved seaward sporadically throughout the trapping period from April to September but their numbers increased markedly in August due to the appearance of underyearlings. The seaward movement of coho fry and smolts and of sticklebacks showed no apparent relationship to either stream discharge or temperature.

Lunar rhythmicity in movement of coho fry and probable fate of fry are discussed within a framework of behavioral and ecologic concepts pertinent to the species' life history in the juvenile stage and relevant to concern for enhancing coho salmon production.

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MASON, J. C. 1975. Seaward movement of juvenile fishes, including lunar periodicity in the movement of coho salmon (*Oncorhynchus kisutch*) fry. J. Fish. Res. Board Can. 32: 2542–2547.

Après émergence, plus de 51,000 alevins de saumon coho (*Oncorhynchus kisutch*) sortirent du ruisseau Lymn. Les mouvements de pointe coïncident avec la nouvelle lune. Ils diminuent graduellement au cours de quatre cycles lunaires, bien que 75% des mouvements de sortie se produisent entre le début d'avril et la mi-juin. La migration des smolts de saumon coho a lieu presque simultanément, atteignant un sommet à la fin de mai, durant une période de marées les plus basses, entre le lever et le coucher du soleil, au temps de la pleine lune. Les épinoches (*Gasterosteus*) descendent sporadiquement vers la mer durant toute la période d'échantillonnage au piège, soit d'avril à septembre, mais leur nombre augmente notablement en août, par suite de l'apparition des jeunes de moins d'un an. Les déplacements vers la mer des alevins et des smolts de saumon coho et ceux des épinoches ne montrent pas de relation avec le débit ou la température du cours d'eau.

L'auteur analyse le rythme lunaire des déplacements d'alevins de saumon coho et le sort probable de ces alevins. Il le fait dans le cadre de concepts de comportement et d'écologie propres aux jeunes stades du cycle biologique de l'espèce et tenant compte de l'intérêt à rehausser la production de saumons coho.

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COHO salmon (*Oncorhynchus kisutch*) typically overseed their spawning grounds in the smaller montane streams of coastal British Columbia. Although dispersal from the redd sites includes a significant upstream component, considerable numbers of the resulting fry move downstream during the subsequent spring and early summer months following their emergence. During May and June, such movement in coastal streams may result in high concentrations of coho fry along the banks and extending seaward from the lower freshwater reaches well into the intertidal zone.

This paper reports on the lunar periodic timing of their downstream movement past a fish weir on Lymn Creek, a small coastal stream on Vancouver Island (Mason 1974a), and comments on their probable fate in the light of behavioral and ecologic concepts pertinent to the species' life history in the rearing stream prior to seaward migration of smolts.

The timing and movement of coho fry is contrasted with that for coho smolts and threespine sticklebacks (*Gasterosteus aculeatus*).

Trapping facility and sampling procedure — The fry fence consisted of a Wolf-type inclined plane of 6-mm wire mesh leading into a collection trough and trap system below the weir. A picket trap for adult salmon, which was reached by passage across the weir floor, was covered with 5-mm wire mesh from spring to autumn in order to retain small fishes ascending the stream.

During operation of the fry fence (March 30—October 16, 1972) the traps were cleared of fish each morning. All fish were identified and counted as to species, coho smolts or yearlings were measured for length and scale samples taken and, apart from composite weekly samples of coho fry (25–50 fish) which were preserved in formalin, the trapped fish were released past the weir in their direction of travel. Yearling coho were identified as presmolts by the retention of high contrast parr marks and flag colors in the dorsal and anal fins.

Results — Coho fry recently emerged from the spawning redds began moving downstream past the fence in early April. Outmovement declined sharply in late August but continued until late autumn. Of the more than 51,000 fry moving seaward, 75% had done so by the middle of June.

Coho fry moved seaward with an evident lunar periodicity, peaks in rate of outmovement coinciding with the phase of the new moon (Fig. 1A) although the peak was somewhat premature in early May and lagged slightly in the ensuing two lunar cycles. Little downstream movement of either fry or smolts occurred during daylight hours although the schedule of trap operations did not permit a strict division of the daily catches into daylight and darkness categories. Periodic trap counts taken throughout several nights in May and June revealed that most fish appeared at the weir between 1000 and 0200.

Factors other than available moonlight, e.g. recruitment rate for emerging fry, may have modified the rate of outmigration but outmovement bore no apparent direct relation to stream discharge or water temperature (Fig. 1D). The variable pattern of cloud cover at night undoubtedly modified intensity of illumination in the lunar rhythm of moonlight hours at night which peak with the full moon (Fig. 1E) but the overall pattern of outmovement remained essentially unchanged.

Time series analysis using information theory after Colwell (1974) was applied to the four cycles encompassing 98% of the total fry output

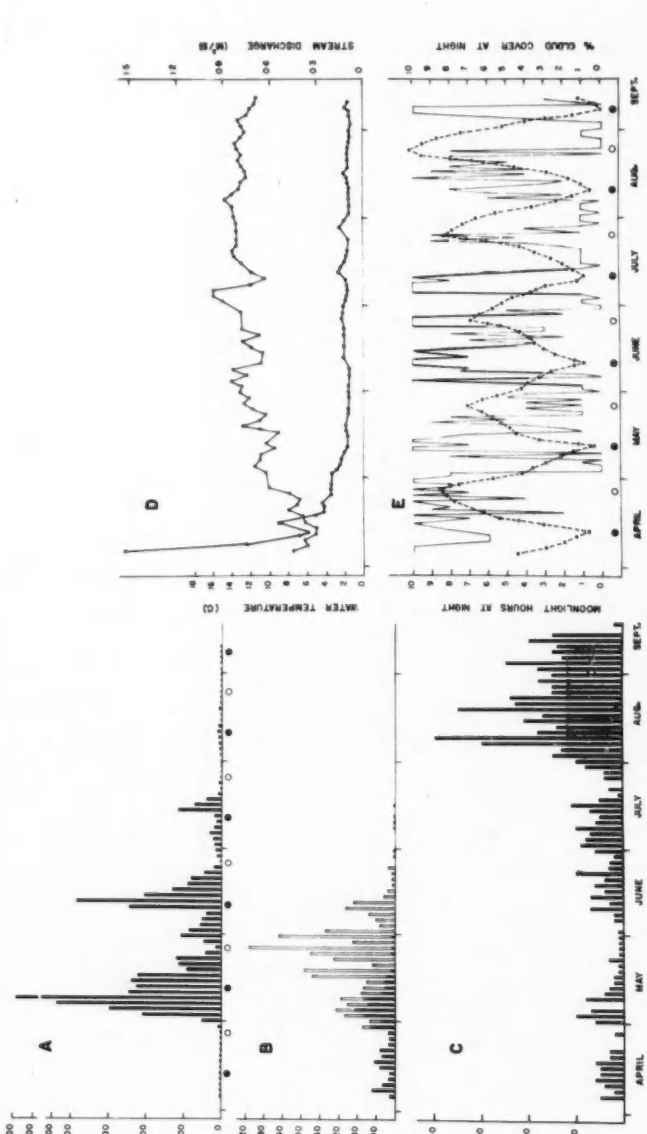

FIG. 1. Downstream movement of fishes at Lymn Creek weir — timing and magnitude of movement and some associated environmental factors. A, Coho salmon (*Oncorhynchus kisutch*) fry movement. Closed circles, new moon; open circles, full moon; B, Presmolt (solid bars) and smolt (open bars) movements; C, Stickleback movements; D, Stream discharge (closed circles) and water temperature (open circles) regimes; E, Lunar timing in hours of moonlight at night (broken line) and cloud cover variability (solid line) during the period of trapping.

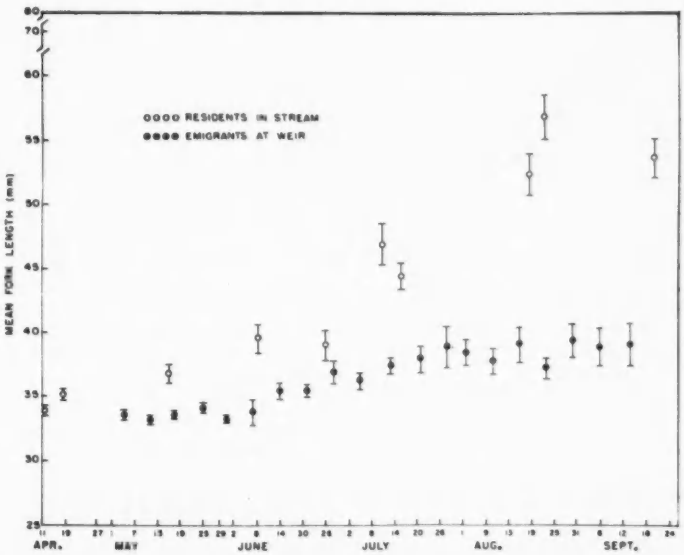

FIG. 2. Onset and progression of size disparity between coho fry resident in Lymn Creek and fry moving downstream that were trapped at the weir. Bars indicate plus or minus 2 SE of the mean.

cast in a frequency matrix. The resulting predictability (P) of 0.64, where P measures the variation among successive periods in periodic outmovement, was not quite significant at the 5% level when tested by the G-statistic. Contingency, which reflects the degree to which time determines state (outmovement) accounted for 67% of the predictability value but its G-statistic was not statistically significant. Although constancy which indicates the degree of departure from common state in time and between cycles contributed only 33% to the predictability value, its G-statistic (68.2 with 14 df, distributed as χ^2) was highly significant.

As a supplemental analysis, the data on coho fry outmovement were marshalled into two series of lunar time intervals: four pairs of periods of high and low moonlight separated by the first and third quarters of the moon. The periods of low moonlight thus involved moonlight at night less than 4 h in duration and were bisected by the new moon. From 75 to 84% of the fry within these paired time intervals moved downstream during the periods of low moonlight.

On the average, outmigrant fry were consistently much smaller than stream residents, the disparity in size increasing progressively with time, so that by September the outmigrants averaged 39 mm compared with 55 mm for resident fry (Fig. 2).

In contrast to the 51,000 fry that moved downstream past the fence, only 360 fry moved upstream from mid-June to late October. These ascending fish were larger on the average than those resident in areas above the weir and produced peaks of movement during mid-July and in late September prior to the first freshet in the autumn.

Yearling coho began moving downstream past the fence in early April but the smolt run proper (some 3000 fish) did not commence in earnest until early May, peaked in late May, and was over by late June (Fig. 1B). As with the coho fry, smolt outmigration bore no apparent relation to stream discharge or water temperature but the run peaked during a period of maximum low tides between sunset and sunrise when the moon was full.

Threespine sticklebacks moved downstream past the fence throughout the trapping period. Their movement was sporadic and of low intensity during the spring and early summer (Fig. 1C) but increased markedly during August and September. Movement during April through June primarily involved yearling and 2-yr-old fish whereas more than 90% of the outmigrants during August and September were underyearlings. As with the coho, their outmigration showed no apparent relationship with either stream flow or temperature.

Discussion and conclusions — Downstream movement of coho fry was examined by Chapman (1962) who concluded that aggressive behavior of social dominants played an important role. When moving fry were placed in natural stream areas devoid of resident fry, most fish assumed residence. His results were corroborated in stream simulators by Mason (1969) who also showed that the size disparity between residents and emigrant fry could be closed rapidly when the latter fry were relieved of intense intra-specific competition.

The propensity of coho fry to cease defending territories and to settle to the stream bottom with the onset of darkness (Hoar 1953) would serve to counteract their displacement by current at night. If, in Lymn Creek, subordinate fry are forced into unsuitable locations for settling during the night, as suggested by Chapman (1962), displacement downstream would probably follow when alternate cues to orientation such as turbulence patterns and point sources of sound (Mason 1974b) are insufficiently stable to allow continued orientation when visual cues are reduced at night and largely determined by available moonlight.

The lunar rhythm in downstream movement of coho fry in Lymn Creek is probably encouraged by the very stable discharge pattern of this stream and its ENE to WSW axis which approximates the path of the moon for nearly 1 km of streambed above the weir site. In less stable streams increased outmovement may coincide with periods of freshet as found by Narver and Andersen (1974). In most coho streams periodic fry losses during fresheting probably exceed those legislated by a density-regulatory behavioral mechanism even though such a mechanism may underlie displacement losses of fry.

From a population dynamics viewpoint, the extent of fry loss from seaward movement in Lymn Creek can be examined, in part, from projections of parental fecundity to survival estimates at emergence and in late summer. Of 540 coho in the 1971 spawning run into Lymn Creek, 222 fish were adult females. By applying an average fecundity of 2310 eggs per female for an adjacent stock reported on by Wickett (1951) and an average survival to emergence of 0.25 drawn from the work of Pritchard (1947) on small streams, the emergent fry population could be approximated as:

$$221 \text{ females} \times 2310 \text{ eggs/female} \times 0.25 = 127{,}628 \text{ fry}$$

The fry population was estimated in late August 1972 as 19,000 fry, by electrofishing using Le-Cren's complete removal technique in 10 representative 50-m sections of stream. Fry survival from emergence in April and May to late August was therefore about 15%. Of the some 108,000 missing coho fry, at least 51,000 or nearly 50% moved downstream past the weir and all but a minor fraction apparently disappeared into the sea.

The ultimate fate of these fry remains obscure. From analysis of scales taken from returning adults which, without exception, showed formation of a freshwater annulus signifying overwinter residence in the stream, we can conclude that most coho fry reaching the estuary fail to survive unless stream residence is reestablished. Less than 1% of the seasonal outmovement in Lymn Creek returned upstream and then not until the late fall. Adjacent streams likewise appear to produce "surplus" fry and I postulate that Lymn Creek fry attempting to invade nearby streams do so with a low probability of success. The inability of coho fry less than 40 mm to osmoregulate adequately at salinities approaching 29 ppt in the marine shallows of Georgia Strait (unpublished data) must severely compound the predicament of such fry, particularly in the estuaries of small streams which drain of sea water during the slack tide.

Large-scale downstream movement of coho fry could be exploited in a salmon enhancement program if the timing of such movement and its probable causative factors are adequately known for specific streams. For example, the lunar periodicity of downstream movement in Lymn Creek could be used to advantage both in design of collection and bypass facilities, and in consideration of logistic support for such culture site operations. Lymn Creek produces from 3000 to 8000 coho smolts annually. The potential increase in production from a small beachfront impoundment adjacent to this stream could exceed natural levels of production by a factor of five yet conserve stock discreteness, given cautious estimates of survival and a supplemental feeding program.

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Analysis of Threshold-Induced Bias Inherent in Acoustic Scattering Cross-Section Estimates of Individual Fish

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WEIMER, R. T., AND J. E. EHRENBERG. 1975. Analysis of threshold-induced bias inherent in acoustic scattering cross-section estimates of individual fish. J. Fish. Res. Board Can. 32: 2547–2551.

During acoustic fish stock assessment surveys, it is often desirable to measure the distribution of the acoustic scattering cross-section of single fish. One of the problems in such measurements is that a threshold in the electronic circuitry discriminates against small fish. This effect is analyzed in detail, and an expression is derived for the threshold-induced bias in the mean scattering cross-section estimate. Results are plotted for a typical set of operating conditions.

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Lors de relevés acoustiques de stocks de poissons en vue d'en évaluer l'abondance, il est souvent désirable de déterminer la distribution de la coupe transversale de dispersion acoustique produite par un seul poisson. Un des problèmes inhérents à cette détermination provient du fait que les circuits électroniques font des distinctions contre les petits poissons. Nous analysons ce phénomène en détail et en arrivons à une formule qui tient compte de l'erreur systématique de seuil dans l'estimation de la coupe transversale dispersante moyenne. Les résultats, applicables à un groupe typique de conditions d'opération, sont représentés graphiquement.

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IN acoustic fish stock assessment surveys employing either echo counters or echo integrators, the acoustic scattering cross section of individual fish is of prime interest. The scattering cross section specifies the amount of acoustic power that is reflected by a fish when it is illuminated by an acoustic signal. Defining I_i as the incident sound intensity at a fish with scattering cross section σ, and I_r as the reflected sound intensity at a distance R from the fish, then I_i and I_r are related by

$$I_r = \left(\frac{\sigma}{4\pi}\right)\frac{I_i}{R^2} \qquad (1)$$

The expression "target strength" is commonly used when referring to a fish's scattering cross section. The target strength, S, of a fish is defined as

$$S = 10 \log_{10}\left(\frac{\sigma}{4\pi}\right) \qquad (2)$$

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The scattering cross section of individual fish is important in acoustic assessment surveys for a number of reasons:

1) In an echo counting system, the average scattering cross section of single fish determines the sampling volume. The squared echo amplitude, E_j, from a single fish can be written as

$$E_j = K_1 \left(\frac{\sigma_j}{4\pi}\right) b^2(\theta_j, \phi_j) \qquad (3)$$

where K_1 is a constant that depends on processing system parameters, σ_j is the scattering cross section of the fish at angular coordinates (θ_j, ϕ_j), and $b(\theta_j, \phi_j)$ is the transducer beam pattern function. Both σ_j and $b(\theta_j, \phi_j)$ are random variables which can be characterized by probability density functions. A fish is counted if its echo amplitude exceeds a threshold, t, which is inherent in the processing system's circuitry. For a given fixed scattering cross section, the threshold level determines the range of angles (and therefore the volume) over which the fish will be detected. When the scattering cross section is not fixed, the average sampling volume can be determined from the distribution of σ.

2) The scattering cross section of fish increases as the length of the fish increases. Therefore, if the scattering cross-section distribution is measured in situ, it can be used for estimating the size distribution within the fish population.

3) In an echo integrator system, the output of the integrator, I, is given by

$$I = K_2 \lambda_o \bar{\sigma} \qquad (4)$$

where K_2 is a constant that depends on processing system parameters, λ_o is the spatial density of fish, and $\bar{\sigma}$ is the mean scattering cross section of individual fish in the population. In order to estimate λ_o from I, both K_2 and $\bar{\sigma}$ must be known or estimated.

Unfortunately, good techniques for estimating $\bar{\sigma}$ are not readily available. One approach has been to make target strength measurements as a function of length, acoustic frequency, and aspect angle in a controlled laboratory experiment. However, since it is difficult to determine the distribution of aspect angles of fish in the open sea, one cannot account for those errors introduced by target strength variation with aspect angle. A second approach for estimating $\bar{\sigma}$, one that avoids this problem, is to measure the target strength in situ by isolating the echoes from single fish and determining their squared echo amplitude, E_j. The beam pattern, $b(\theta_j, \phi_j)$, can be extracted either indirectly from the empirically determined distribution of the E_j (Craig and Forbes 1969) or directly from each E_j (Ehrenberg and Lytle 1973; Dunn 1973; Ehrenberg 1974).

Problem definition — One major problem with the techniques for estimating in situ target strength is that they discriminate against small targets; this effect is inherent in the processing system's circuitry. Single targets are recognized only if their squared echo amplitude exceeds a threshold level t, which is determined by the noise at the output of the processing system. That is, if

$$E_j = K_1 \left(\frac{\sigma_j}{4\pi}\right) b^2(\theta_j, \phi_j) > t \qquad (5)$$

then a single echo is recognized. The t threshold and maximum expected signal, E_{max}, determine the system's dynamic range, R, which is defined as

$$R = E_{max}/t \qquad (6)$$

It can be seen that as a result of t, a fish with a larger scattering cross section will be detected over a wider range of angles than will a fish with a smaller scattering cross section. Consequently, the effect of t on the mean scattering cross-section estimate, $\hat{\sigma}_t$, will be to bias it high. The basic in situ target strength estimation technique described by Ehrenberg and Lytle (1973) and Ehrenberg (1974) can be modified to minimize this effect. The modification is mentioned by Ehrenberg (1974) and has been analyzed in detail by Ehrenberg and Weimer (1974).

This note presents a general derivation for the threshold-induced bias inherent in in situ mean scattering cross-section estimates. To illustrate the effects of this threshold, plots of the normalized error in $\hat{\sigma}_t$ as a function of t are presented for a modeled hydroacoustic system using a circular piston transducer, and assuming that the fish are uniformly distributed in space with normally distributed target strengths. Finally, results are presented that show the effects of t on target strength density plots made from biased sequences of echoes.

Analysis — The estimate for the mean scattering cross section of a fish population obtained from processing N single echoes exceeding the threshold is given by the sample mean

$$\hat{\sigma}_t = \frac{1}{N} \sum_{j=1}^{N} \sigma_{tj} \qquad (7)$$

$$= \frac{1}{N} \sum_{j=1}^{N} \frac{4\pi E_{tj}}{b^2(\theta_j, \phi_j)} \qquad (8)$$

where E_{tj} are squared echo levels exceeding the threshold t. The bias of $\hat{\sigma}_t$ is defined as

$$B(\hat{\sigma}_t) \triangleq E[\hat{\sigma}_t] - \bar{\sigma} \qquad (9)$$

in which $E[\hat{\sigma}_t]$ is the expected value of $\hat{\sigma}_t$ and $\bar{\sigma}$ is the actual mean scattering cross section of the fish population. To obtain an explicit expression

for $B(\hat{\sigma}_t)$ in terms of t, it is first necessary to compute the probability density function of σ conditioned on $E > t$. For notational convenience in the following derivation, define two new random variables, X and Y,

$$X \triangleq \frac{\sigma}{4\pi} \tag{10}$$

and

$$Y \triangleq b^2(\theta, \phi) \tag{11}$$

The threshold condition, equation 5, may then be rewritten as

$$E_j = X_j\, Y_j > t \tag{12}$$

in which the constant K_1 has been set equal to one for notational convenience and without loss of generality. The random variable X may take on values in the interval $(0, +\infty)$, while Y is confined to the interval [0, 1]. The conditional density function of X, $f_{X|t}(x|xy > t)$, may be computed by finding the conditional distribution function, $F_{X|t}(x|xy > t)$ and then differentiating it. That is,

$$f_{X|t}(x|xy > t) = \frac{d}{dx}[F_{X|t}(x|xy > t)] \tag{13}$$

Writing the definition of the conditional distribution function in terms of probabilities,

$$F_{X|t}(x|xy > t) = P(X < x|XY > t) \tag{14}$$

Bayes' Rule can be applied to rewrite this as

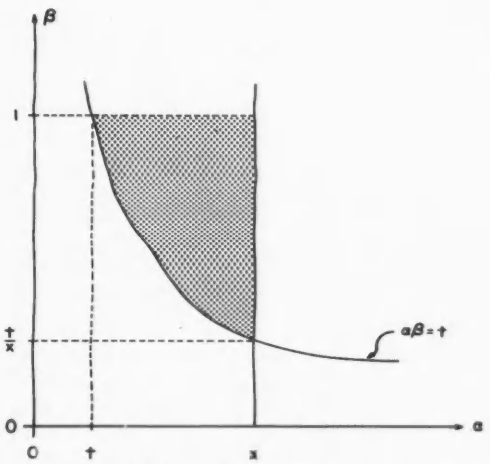

FIG. 1. The shaded region represents the area over which the joint density function, $f_{X,Y}(\alpha,\beta)$, must be integrated to compute $P(X < x, XY > t)$.

$$F_{X|t}(x|xy > t) = \frac{P(X < x, XY > t)}{P(XY > t)} \tag{15}$$

Substituting equation 15 into equation 13 gives

$$f_{X|t}(x|xy > t) = \frac{1}{P(XY > t)} \cdot \frac{d}{dx}[P(X < x, XY > t)] \tag{16}$$

Figure 1 shows the appropriate region over which the joint density function, $f_{X,Y}(x, y)$, must be integrated to compute $P(X < x, XY > t)$. The result is

$$P(X < x, XY > t) = \begin{cases} 0 & \text{for } x \le t \\ \int_{t/x}^{1} \int_{t/\beta}^{x} f_{X,Y}(\alpha, \beta)\, d\alpha\, d\beta & \text{for } x > t \end{cases} \tag{17}$$

In a similar manner,

$$P(XY > t) = \int_{t}^{\infty} \int_{t/\alpha}^{1} f_{X,Y}(\alpha, \beta)\, d\beta\, d\alpha \tag{18}$$

It can be assumed that σ and $b^2(\theta, \phi)$ are statistically independent. Therefore X and Y are also independent random variables and their joint density function is simply the product of their two individual density functions,

$$f_{x,y}(x, y) = f_x(x)\, f_y(y) \tag{19}$$

Equations 17 through 19 may be substituted into equation 16 and the differentiation performed to give a final general expression for the conditional density function of X (and therefore σ),

$$f_{X|t}(x|xy > t) = \begin{cases} 0 & \text{for } x \le t \\ \dfrac{f_X(x) \displaystyle\int_{t/x}^{1} f_Y(\beta)\, d\beta}{\displaystyle\int_{t}^{\infty} \int_{t/\alpha}^{1} f_X(\alpha) f_Y(\beta)\, d\beta\, d\alpha} & \text{for } x > t \end{cases} \tag{20}$$

The specification of the two individual density functions, $f_X(x)$ and $f_Y(y)$, now completely determines $f_{X|t}(x|xy > t)$.

With the explicit expression for $f_{X|t}(x|xy > t)$ given by equation 20, $B(\hat{\sigma}_t)$ can now be computed. We first note that

$$B(\hat{\sigma}_t) = 4\pi\, B(\hat{X}_t) \tag{21}$$

where

$$\hat{X}_t = \frac{1}{N} \sum_{j=1}^{N} X_{tj} \tag{22}$$

and $X_{t1}, X_{t2}, \ldots, X_{tN}$ are independent and identically distributed on the conditional density function $f_{X|t}(x|xy > t)$. Equation 21 can now be written as

$$B(\hat{\sigma}_t) = \sigma_{\pi} (E[\hat{X}_t] - \bar{X}) \quad (23)$$

in which

$$E[\hat{X}_t] = \int_0^{\infty} x f_{X|t}(x|xy > t)dx \quad (24)$$

and

$$\bar{X} = \int_0^{\infty} x f_X(x)dx = \frac{1}{4\pi} \int_0^{\infty} \sigma f_{\sigma}(\sigma)d\sigma \quad (25)$$

The function $f_{\sigma}(\sigma)$ is the actual scattering cross-section density for the fish population.

With the specification of $f_X(x)$ and $f_Y(y)$, the bias in the mean scattering cross-section estimate can be computed as a function of the processing system's inherent threshold.

Application — In order to illustrate the effects of the t threshold on the mean scattering cross-section estimate, the following model for an acoustic system and fish population was investigated.

1) The fish are uniformly distributed in the volume of water. Ehrenberg (1972) has shown that, for a uniform fish distribution, the density function for the directivity factor of a circular piston transducer is

$$f_Y(y) = \begin{cases} (1-c)y^{-c} & \text{for } 0 \le y \le 1 \\ 0 & \text{otherwise} \end{cases} \quad (26)$$

where c is a constant depending on the properties of the particular transducer used.

2) The target strengths of the fish are normally distributed with mean μ and standard deviation ρ. This assumption infers that the density function $f_X(x)$ is given by the lognormal distribution,

$$f_X(x) = \begin{cases} 0 & \text{for } x < 0 \\ \dfrac{10 \log_{10} e}{\sqrt{2\pi}\rho x} \exp\left[-(10 \log_{10} x - \mu)^2/(2\rho^2)\right] & \text{for } x \ge 0 \end{cases} \quad (27)$$

Under these two assumptions, the conditional density function for X, equation 20, may be written as

$$f_{X|t}(x|xy > t) = \begin{cases} 0 & \text{for } x \le t \\ \left[1 - \left(\dfrac{t}{x}\right)^{(1-c)}\right] \dfrac{f_X(x)}{D} & \text{for } x > t \end{cases} \quad (28)$$

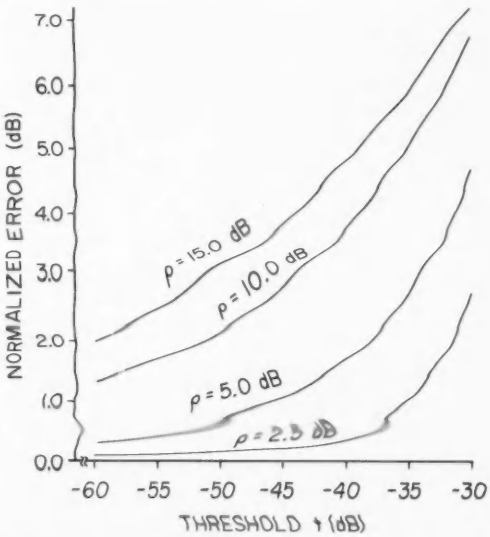

FIG. 2. Normalized error (10 $\log_{10}$ $(E[\hat{X}_t]/\bar{X})$) vs threshold t for the case of normally distributed target strengths with mean $\mu = -30$ dB and SD $= \rho$.

in which $f_X(x)$ is given by equation 27 and

$$D = \int_t^{\infty} \left[1 - \left(\frac{t}{x}\right)^{(1-c)}\right] f_X(x)dx \quad (29)$$

The expected value of the biased estimator $\hat{X}_t$ is then

$$E[\hat{X}_t] = \frac{1}{D} \int_t^{\infty} x \left[1 - \left(\frac{t}{x}\right)^{(1-c)}\right] f_X(x)dx \quad (30)$$

and the true mean of X obtained from equations 25 and 27 is

$$\bar{X} = 10^{[\mu/10 + \rho^2/(200 \log_{10} e)]} \quad (31)$$

Using numerical techniques to evaluate the integrals, plots of the normalized error (defined as 10 $\log_{10}$ $(E[\hat{X}_t]/\bar{X})$) as a function of the threshold t were made for a mean value of $\mu = -30$ dB, SD of 2.5, 5.0, 10.0, and 15.0 dB, and a transducer constant of $c = 0.86$ (corresponding to a 12-degree beamwidth circular transducer). The resulting curves are shown in Fig. 2. Note that for fish populations with large standard deviations, it is especially important to have a large dynamic range in the receiver to keep the value of t small and reduce the bias in the mean scattering cross-section estimate.

In addition to biasing the mean scattering cross-section estimate, the threshold t will also affect

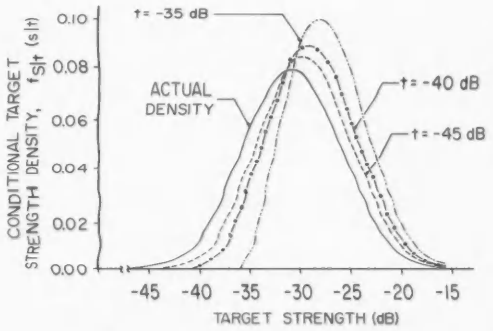

FIG. 3. *Conditional target strength densities for* three representative values of t. The actual density is normal with mean $\mu = -30$ *d*B and SD $\rho = 5$ *d*B.

the shape of target strength density plots made from biased sequences of echoes. To illustrate this effect, the conditioned scattering cross-section density, $f_{X|t}(x|xy > t)$ in equation 28, can be *transformed to obtain the conditional target* strength density, $f_{S|t}(s|t)$. Note from equation 2 that

$$X = 10^{s/10} \tag{32}$$

The two conditional density functions are related by

$$f_{S|t}(s|t)\, ds = f_{X|t}(x|xy > t)\, dx \tag{33}$$

or equivalently,

$$f_{S|t}(s|t) = f_{X|t}(x|xy > t)\, \frac{dx}{ds} \tag{34}$$

Substituting equation 32 into equation 34 and *performing the differentiation yields*

$$f_{S|t}(s|t) = \left(\frac{\ln 10}{10}\right) \times f_{X|t}(x|xy > t) \tag{35}$$

Figure 3 shows $f_{S|t}(s|t)$ for three values of t when the actual target strengths of the individual fish are normally distributed with mean $\mu = -30$ *d*B and SD $\rho = 5$ *d*B. Again, the need is evident for a large dynamic range in the receiver to keep the value of t small and reduce the discrepancies between the actual and conditional target strength densities.

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Zinc Uptake by Atlantic Salmon (*Salmo salar*) Exposed to a Lethal Concentration of Zinc at 3, 11, and 19 C[1]

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HODSON, P. V. 1975. Zinc uptake by Atlantic salmon (*Salmo salar*) exposed to a lethal concentration of zinc at 3, 11, and 19 C. J. Fish. Res. Board Can. 32: 2552–2556.

Atlantic salmon (*Salmo salar*) acclimated to 3, 11, and 19 C took up high concentrations of zinc in gill tissues during exposures to a nominal 14 mg/liter of zinc. The rate of zinc uptake, as shown by ^{65}Zn tracer, increased from a minimum at 3 C to a maximum at 19 C. In fish killed by zinc exposure, zinc concentrations in gills were highest at 19 C and lowest at 3 and 11 C.

HODSON, P. V. 1975. Zinc uptake by Atlantic salmon (*Salmo salar*) exposed to a lethal concentration of zinc at 3, 11, and 19 C. J. Fish. Res. Board Can. 32: 2552–2556.

Des saumons atlantiques (*Salmo salar*) acclimatés à des températures de 3, 11 et 19 C captèrent de fortes concentrations de zinc dans leurs tissus branchiaux 8 h après exposition à une concentration nominale de 14 mg/litre de zinc. Le taux de captation du zinc, tel qu'indiqué par marquage au ^{65}Zn, augmente d'un minimum à 3 C à un maximum à 19 C. Chez des poissons tués par exposition au zinc, les concentrations de zinc dans les branchies sont maximales à 19 C et minimales à 3 et 11 C.

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WATER temperature modifies the lethal effects of heavy metals to fish, principally by shortening survival times as temperatures increase (Burton et al. 1972a; Cairns et al. 1971; Hodson and Sprague 1975; Lloyd 1960; MacLeod and Pessah 1973; Sprague 1964; Sumner and Wells 1935). Hodson and Sprague (1975) also found that the 2-wk median lethal concentration (LC50) of zinc was higher for Atlantic salmon (*Salmo salar*) acclimated to 19 C than for salmon acclimated to 3 or 11 C.

Studies of gill pathology (Lloyd 1960; Skidmore and Tovell 1972), oxygen uptake (Skidmore 1970), osmoregulation (Lewis and Lewis 1971; Skidmore 1970), and anaerobic metabolism (Burton et al. 1972b) of acutely zinc-poisoned fish suggest that fish die as a result of damage to gills.

Variation of zinc toxicity with temperature may be due to a difference in sensitivity of fish gills to zinc or to the rate at which gill damage occurs. Therefore, zinc uptake by gills of fish during a lethal zinc exposure was measured to determine whether temperature affects the rate of zinc uptake or the concentration of zinc at death.

Materials and methods — Experimental design — Live fish were removed at increasing intervals from a lethal solution of zinc sulphate and their gills analyzed for zinc concentrations. The design was a 3 × 5 factorial with three exposure temperatures and five sampling times for live fish. Fish that died were also sampled and an extra group of fish was exposed to zinc to increase the sample size of dead fish. The experiment was repeated on 2 successive days.

Fish and acclimation conditions — Atlantic salmon were acclimated in hard water to 3.0 ± 0.06, 11 ± 0.13, and 19 ± 0.13 C as described in Hodson and Sprague (1975). Minimum acclimation time was 65 days. Average oxygen concentrations varied from 7.8 mg/liter at 19 C to 13 mg/liter at 3 C and exceeded 85% of air saturation. Total hardness averaged 349 mg/liter (as $CaCO_3$) and pH ranged from 7.6 to 8.1. Salmon were 13–14 mo old and averaged 5.6 g at 3 C. This was about ⅓ the weight of those at 11 and 19 C due to low growth rates (Hodson 1974).

Experimental procedure — Zinc was measured in gills of salmon exposed to a nominal 14 mg/liter of Zn^{++}. This acutely lethal concentration caused rapid mortality of salmon (Hodson and Sprague 1975) and allowed sampling to be completed within 16 h. Approximately 1 μC/liter of ^{65}Zn ($\simeq 1 \times 10^{-5}$ mg/liter) was included as a tracer since it should behave like stable zinc in biological systems (Polikarpov 1966).

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TABLE 1. Means and 95% confidence limits of zinc concentrations in exposure tanks. Sample size for ^{65}Zn = 22; stable zinc = 6.

Zinc concn	Temp (C) 3	11	19
^{65}Zn (cpm/5 ml)[a]	390.4±5.6	406.3±8.9	343.2±6.9
Stable zinc (mg/liter)	12.25±0.30	12.26±0.32	10.90±0.49
(μg/5 ml)	61.3	61.3	54.5
Specific activity (cpm/μg Zn)	6.37	6.63	6.30
Relative concn of stable zinc	1.14	1.18	1.00

[a]Counts per minute per 5.0-ml sample.

The advantages of a tracer were a low detection limit for zinc, and the zinc analyses did not include stable zinc normally present in tissues of unexposed fish.

Fish were exposed to zinc in a continuous-flow bioassay system (Hodson 1974). One fail-safe toxicant meter at each temperature delivered 250 ml/min of zinc solution to each of two 20-liter exposure tanks. This equalled 360 liters per tank per day or more than 2 liters/g of fish per day. The 95% replacement time was 3 h.

Concentrations of ^{65}Zn in toxicant stock solutions were adjusted to give a similar specific activity (ratio of tracer to stable zinc) at all temperatures. During the experiment, water was sampled for measurement of ^{65}Zn by gamma scintillation counting and stable zinc by atomic absorption spectroscopy.

Twenty-four salmon at each temperature were selected in a random sequence and exposed in groups of 12, at their acclimation temperature, to the lethal solution of zinc. In addition, 12 control fish were exposed to uncontaminated water at 11 C.

One fish was removed from each tank at 0.5, 1, 2, 4, and 8 h from the start of exposure. Each fish was killed by a blow on the head, placed in a numbered plastic bag, and frozen at −20 C. Fish killed by zinc were similarly stored within 10 min of death.

The experiment was repeated the following day. In addition, extra salmon were killed by exposure to zinc at all three temperatures to increase the sample size of dead fish.

Fish were thawed individually and in a random sequence. Gills were placed in numbered vials (15 × 110 mm), weighed, and sulphuric acid (75%) was added to a total volume of 5.0 ml per vial. Vials were capped and shaken once daily for several days to aid sample digestion.

Radioactivity of each gill and water sample was measured with the same efficiency in a gamma scintillation counter (Nuclear Chicago, Model 4233). Preset counts of 10,000 or preset times of 40 min limited each count and a standard and blank were measured every 50 samples. Average background was used to calculate net sample counts and counts per minute. Only net sample counts with a coefficient of variation of less than 10% were converted to counts per minute after correction for decay. Net sample counts with a coefficient of variation greater than 10% were too close to background counts to be considered significant.

Total nitrogen concentration for all radioactive gill samples was measured by the standard AOAC Kjeldahl method (2.044; Horwitz 1965). Results were subsequently expressed as counts per minute per gram nitrogen (cpm/g N), to avoid inaccuracies due to expressing results on a wet weight basis. It was assumed that tissue protein metabolism and nitrogen content could not change significantly during an 8-h exposure. Moisture content, however, might vary with zinc damage to gills or with evaporative loss due to freezing.

Activity in gills (cpm/g N) is used throughout this paper as an index of amount of stable zinc taken up by gills. Data were not converted to concentrations of stable zinc because background levels of stable zinc in gills prevented a check on specific activity (ratio of ^{65}Zn to stable zinc).

Nonhomogenous variance of activities in gills of live fish (Bartlett's Test; Steel and Torrie 1960) was corrected by a $\log_{10}$ transformation. Variation of ($\log_{10}$ activity in gills) with time and temperature was calculated by factorial analyses of variance. Equations describing this variation were derived by multiple regressions of the transformed data on factors shown by the analysis of variance to be significant. Differences were tested at the 95% confidence level.

Analyses of variance for unequal sample size measured variation of activity in gills with temperature at death. Partitioning the temperature sums of squares gave a statistical comparison of treatment means at the 95% confidence level.

Results and discussion — Specific activities in exposure tanks were similar at all temperatures (Table 1). Measured zinc concentrations were less than nominal concentrations. Also, total zinc concentrations at 3 and 11 C exceeded those at 19 C by factors of 1.14 and 1.18, respectively. Consequently, zinc concentrations in fish from 3 and 11 C were adjusted to the 19 C level by dividing by 1.14 and 1.18, respectively. The

slightly lower concentrations of zinc in test solutions at 19 C were probably due to a poorly calibrated doser. Adjustment of results to the 19 C level was based on the assumption that zinc uptake should be proportional to the zinc concentration in water (Polikarpov 1966). Therefore, activities in gills of fish at 3 and 11 C were relatively high compared to those at 19 C. The statistical analyses gave the same results with and without adjustment. However, the adjustment ensured that the comparisons were the same at all temperatures. Control fish in uncontaminated water contained no detectable radioactivity. Therefore, there was no detectable background contamination.

Rate of zinc uptake in gill tissue — Salmon at 19 C took up ^{65}Zn in gill tissue faster than those at 11 C and 3 C (Fig. 1). The analysis of variance indicated that activity in gills was significantly controlled by: 1) a curvilinear temperature effect; 2) a curvilinear time effect; 3) a linear temperature–time effect; and 4) a day effect (Hodson 1974). In other words, activity in gills increased both with temperature and the square of temperature (Fig. 1) so that the greatest increase occurred between 11 and 19 C. The curvilinear time effect describes a significant increase, decrease, and subsequent increase of activity in gills with time at all temperatures. The time–temperature interaction indicates significantly nonparallel regression lines at each temperature; that is, rate of ^{65}Zn uptake accelerated with temperature and time (Fig. 1).

This supports the hypothesis that warm-acclimated fish die faster because zinc is taken up more rapidly to a lethal concentration. A possible explanation is that the higher ventilation rates of warm-acclimated fish (Belding 1929; Heath 1973; Hughes and Roberts 1970; Spitzer et al. 1969) maintain a greater water flow across the gills than in cold-acclimated fish. Consequently, zinc availability and uptake at the gill surface is greater. There is no evidence that warm-acclimated fish die faster because of greater sensitivity to a given concentration of zinc in the gills.

Acceleration of zinc uptake with temperature may result from gradual damage to gill tissue. Gill damage causes tissue hypoxia (Burton et al. 1972a) that would induce higher ventilation rates (Hughes 1973). Gill damage might also inhibit transferral of zinc from gills by slowing diffusion.

The "bump" in curves of activity in gills vs. time at 1 h (Fig. 1) may also have resulted from increased ventilation rates due to handling of fish (Stevens 1972) at time 0. As fish calmed down, transferral of zinc from the gills may have exceeded uptake until gill damage promoted uptake and inhibited transfer.

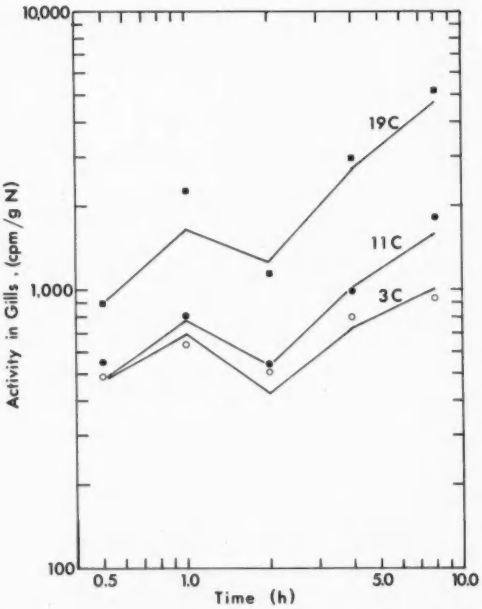

FIG. 1. The increase with time of activity in gill tissue of zinc-exposed Atlantic salmon (*Salmo salar*) at three temperatures.

$$\text{Activity in gills} = \text{Antilog}\,[2.8967 - .0241\,Te + .0022\,Te^2 - .9733\log_{10}Ti - 1.4666\,(\log_{10}Ti)^2 + 9.196\,(\log_{10}Ti)^3 - 6.9054\,(\log_{10}Ti)^4 + .0206\,Te\log_{10}Ti]$$

Where Te = temperature, Ti = time, and multiple $R^2 = 77.3\%$.

The lines in the graph are predicted by the equation. The points are the measured means at each temperature and are included to indicate how the predicted lines fit the actual data.

The day effect indicated that activity in gill tissues at all temperatures was significantly higher on day 1 than on day 2. This suggests a greater initial uptake on day 1. Since the fish on day 2 were disturbed by experimental activity for 2 successive days, they may have acclimatized to the disturbance. Consequently they would be less active, have a lower respiration rate, and take up zinc more slowly.

There were no significant day-treatment interactions in the analysis of variance. In other words, the shape of the curves of Fig. 1 are identical on day 1 and day 2. The equation in Fig. 1 accounts for 77.3% of variability in the data but does not include the day effect. Days account for only a further 3.4%. The "predicted" lines of Fig. 1 would correspond to the means more closely if the day effect were included in the equation. Be-

cause the experiment was designed to remove the day effect from the error variance, differences due to temperature and time were identified with greater certainty.

Total zinc uptake in gill tissue at death — Activity of zinc in gills was 3250, 3060, and 8130 cpm/g N at 3, 11, and 19 C, respectively. The standard deviation, as estimated by the analysis of variance, was 410 cpm/g N. Therefore, the activity in gills at 19 C was significantly different from that at 3 and 11 C. There was a significant day effect but no day–temperature interaction.

This supports the hypothesis that, at higher temperatures, higher concentrations of zinc in gill tissues are required to cause gill damage and death. In fact, activity in gills at death is related to temperature in the same way as lethal thresholds measured by Hodson and Sprague (1975). Lethal thresholds were 3.5 and 3.6 mg/liter at 3 and 11 C, respectively, and 5.4 mg/liter at 19 C (Hodson and Sprague 1975). In other words, zinc concentrations in gills of dead fish appear closely related to lethal threshold concentrations.

This information does not support the hypothesis that higher lethal thresholds of warm-acclimated fish result from a faster removal of zinc from gills. If this were true, all fish would have died when zinc concentrations in gills reached a common lethal level.

The high concentrations of zinc in warm-acclimated fish gills suggests that the physiological response to intoxication of warm-acclimated fish differs from that of cold-acclimated fish. One possibility is that resistance to inhibition of an enzyme in the gills may increase with temperature. An alternative is that tissue hypoxia or a breakdown in osmoregulation induced by gill damage has less impact on the fish at higher temperatures. The result would be prolonged survival of the fish and a greater total uptake of zinc by gills.

There is a possibility that a proportion of ^{65}Zn uptake may represent exchange for stable zinc in gill tissues. However, Mount (1964) showed that most zinc in gills of unexposed fish was present in the hard, cartilagenous gill arches. During an exposure, zinc concentrations increased principally in the soft tissues. Therefore, it would appear that zinc already in gill tissues is incorporated into the cartilage as the fish grows and is probably unavailable for exchange with ^{65}Zn.

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Acute Toxicity to Juvenile Rainbow Trout (*Salmo gairdneri*) of Naturally Occurring Insect Juvenile Hormone Analogues

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LEACH, J. M., A. N. THAKORE, AND J. F. MANVILLE. 1975. Acute toxicity to juvenile rainbow trout (*Salmo gairdneri*) of naturally occurring insect juvenile hormone analogues. J. Fish. Res. Board Can. 32: 2556–2559.

Median lethal concentrations (LC50) of four insect juvenile hormone analogues (IJHA) for juvenile rainbow trout (*Salmo gairdneri*) were measured in bioassays with 4-h solution renewal. The most toxic compound tested was $\Delta^{4'}$-dehydrojuvabione, with a 48-h LC50 of 1.4 mg/liter and an estimated 96-h LC50 of approximately 0.8 mg/liter. Juvabione, epimeric mixtures of dihydrojuvabiones, and epimeric mixtures of juvabiols had 96-h LC50's of 1.5, 1.8, and 2.0 mg/liter, respectively. All fish, including those that survived for 96 h, became lethargic soon after exposure to solutions of the IJHA, and their skins darkened. The toxicants were unstable in aqueous solution.

LEACH, J. M., A. N. THAKORE, AND J. F. MANVILLE. 1975. Acute toxicity to juvenile rainbow trout (*Salmo gairdneri*) of naturally occurring insect juvenile hormone analogues. J. Fish. Res. Board Can. 32: 2556–2559.

Les concentrations létales médianes (CL50) de quatre analogues d'hormone juvénile d'insectes (AHJI) à l'endroit de jeunes truites arc-en-ciel (*Salmo gairdneri*) ont été déterminées par essais biologiques, avec renouvellement de la solution après 4 h. Parmi les composés essayés, la $\Delta^{4'}$-déshydrojuvabione est le plus toxique, d'une CL50 après 48 h de 1.4 mg/litre et une CL50 après 96 h estimée à environ 0.8 mg/litre. La juvabione, les mélanges épimériques de dihydrojuvabiones et les mélanges épimériques de juvabiols ont des CL50 après 96 h de 1.5, 1.8 et 2.0 mg/litre, respectivement. Tous les poissons, y compris ceux qui survivent pendant 96 h, deviennent léthargiques peu après exposition aux solutions d'AHJI, et leur peau devient plus foncée. Les toxiques sont instables en solution aqueuse.

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MECHANICAL pulping effluents produced during the manufacture of groundwood pulp from mixtures of spruce, pine, and fir contain juvabione, juvabiol (Fig. 1), and $\Delta^{1'}$-dehydrojuvabione as major constituents of neutral fractions that are acutely toxic to fish (Leach and Thakore 1974a). These compounds, and the related $\Delta^{4'}$-dehydro-

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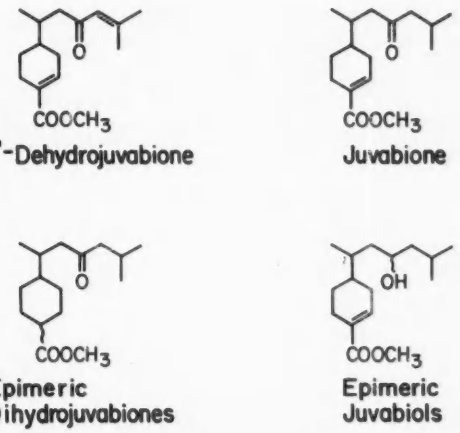

FIG. 1. Structural formulae of insect juvenile hormone analogues (IJHA) tested for toxicity to fish.

juvabione and *cis*-dihydrojuvabione (Fig. 1), are constituents of Douglas fir (*Pseudotsuga menziesii*), alpine fir (*Abies lasiocarpa*), and/or balsam fir (*Abies balsamea*) (Bowers et al. 1966; Rogers et al. 1974; Manville 1975). Most are insect juvenile hormone analogues (IJHA) that have been tested for use in the control of various forest pests.

Since pulpmill discharges and insect spraying practices could result in abnormal concentrations of IJHA in surface waters, their toxicity to fish is of concern. Thus, 96-h median lethal concentrations (LC50) of four naturally occurring IJHA (Fig. 1) were measured for rainbow trout (*Salmo gairdneri*).

Materials and methods — Juvabione and $\Delta^{4'}$-dehydrojuvabione were purified from extracts of balsam fir (Manville 1975). Epimeric juvabiols were isolated from balsam fir (E. P. Swan and J. F. Manville unpublished results) or were synthesized by sodium borohydride reduction of juvabione. Either procedure gave a similar epimeric mixture as determined by gas–liquid chromatography (glc; POLY-A 101A on 100–120 mesh Gas Chrom Q). Dihydrojuvabiones (1:2 mixtures of *cis*- and *trans*-isomers) were obtained by hydrogenating juvabione or $\Delta^{4'}$-dehydrojuvabione in methanol over Pd on charcoal with H_2 (24 psi) and were distilled under vacuum to eliminate heavy metal contamination. The purity of each compound or epimeric mixture was greater than 95% by thin layer chromatography (tlc; silicic acid; petroleum ether/diethyl ether, 3:1) and by glc. Individual contaminants comprised less than 2% of any sample.

Toxicity was measured at 8 ± 1 C using rainbow trout, approximately 70 days old, acclimated at the same temperature. Mean body weight was 0.8 ± 0.35 g and mean fork length was 4.0 ± 0.57 cm. Each test solution held 10 fish and was contained in two 3-liter aquaria (surface area 430 cm^2) lined with removable fine-mesh fiberglass baskets. The dilution water was dechlorinated Vancouver city tapwater (pH 6.8, specific conductance 20 μmho/cm, EDTA hardness 7.7 mg $CaCO_3$/liter, alkalinity 6.0 mg $CaCO_3$/liter), and solutions were adjusted to pH 7.0 ± 0.2 with 20 ml McIlvaine's buffer (Anon 1947). Dissolved oxygen was maintained at 10 ± 1 mg/liter by minimal aeration.

Stock solutions of each toxicant were prepared as follows and stored at 8 C. The compound (250 mg) was dissolved in methanol (5 ml), Miranol J2M dispersant (3.8 g) was added, and the solution was bulked to 50 ml with dechlorinated water.

Preliminary tests were carried out using glc analysis to determine the stability of IJHA under bioassay conditions. Based on the results obtained, fish were transferred to freshly prepared solutions every 4 h, giving a volume of 4.5 liters/g per day. Periodic instantaneous solution replacement in bioassays of kraft pulpmill effluent has been shown to give toxicity data similar to those obtained by continuous-flow techniques (Walden et al. 1975). Solutions of dehydroabietic acid, used as a reference toxicant, and controls with dechlorinated water containing methanol, Miranol J2M, and buffer, were also renewed at 4-h intervals. Bioassays were observed for 96 h and fish were removed when they lost equilibrium permanently.

Toxicant concentrations were measured as follows during the bioassays. Aliquots (2 ml) of stock solutions and aliquots (1 liter) of bioassay solutions after 4 h exposure of fish were treated with saturated Na_2SO_3 solution (50 ml), and the IJHA were extracted with diethyl ether. Solvent was evaporated from the extracts, heptadecanoic acid (0.75 mg) in ether (3 ml) was added as an internal standard, and the solutions were methylated with diazomethane. IJHA concentrations were measured by glc (1.8-m × 0.32-cm stainless steel column containing 10% SILAR-10C on 60–80 mesh Gas Chrom W; 150–220 C at 4 C/min; He flowrate 66 ml/min) using response factors determined for the pure compounds. Recoveries of IJHA from aqueous solutions were 100 ± 6%.

Effective toxicant concentrations during bioassays were calculated from the average of the mean initial and mean final concentrations measured at the time of solution replacement (Brown et al. 1969).

Results and discussion — In the 4 h between renewal of bioassay solutions, the concentration of epimeric juvabiols decreased by 14 ± 8%, and concentrations of juvabione, $\Delta^{4'}$-dehydrojuvabione, and epimeric dihydrojuvabiones decreased by approximately 35% (Table 1). LC50s of the IJHA for juvenile rainbow trout (Table 2) were obtained by standard graphical interpolation (American Public Health Association 1971). The approximate 96-h LC50 of $\Delta^{4'}$-dehydrojuvabione was extrapolated from median lethal times (LT50) of fish, since insufficient material was available for direct measurement. Dehydroabietic acid, used

TABLE 1. Initial, final, and effective (mean) concentrations of insect juvenile hormone analogues in bioassay solutions.

IJHA	Initial concn (*mg/liter*) Mean[a]	Initial concn (*mg/liter*) Range	Final concn (*mg/liter*) Mean[a]	Final concn (*mg/liter*) Range	Effective concn (*mg/liter*)
$\Delta^{4'}$-Dehydrojuvabione	1.3 (3)	1.2–1.3	1.0 (4)	0.8–1.1	1.2
	2.0 (1)	—	1.3 (3)	1.1–1.5	1.7
Juvabione	1.65 (2)	1.5–1.8	1.2 (5)	1.1–1.3	1.4
	2.1 (3)	1.9–2.4	1.3 (4)	1.1–1.6	1.7
Dihydrojuvabiones	1.85 (2)	1.8–1.9	1.3 (5)	1.1–1.5	1.6
	2.2 (3)	2.0–2.4	1.3 (4)	1.1–1.6	1.8
Juvabiols	1.75 (2)	1.6–1.9	1.5 (5)	1.4–1.5	1.6
	2.2 (3)	2.1–2.3	2.0 (4)	1.7–2.1	2.1

[a]*Number of determinations in brackets.*

TABLE 2. Toxicities of insect juvenile hormone analogues to rainbow trout (*Salmo gairdneri*).

IJHA	Concn in bioassay solution (*mg/liter*)	LT50 of fish (*min*)	Fish surviving for 96 h (%)	96-h LC50 (*mg/liter*)
$\Delta^{4'}$-Dehydrojuvabione	1.2	3200	10	0.8[a]
	1.7	2450	0	
Juvabione	1.4	—	60	1.5
	1.7	4250	40	
Dihydrojuvabiones	1.6	—	80	1.8
	1.8	3500	50	
Juvabiols	1.6	—	80	2.0
	2.1	3300	40	

[a]*Extrapolated from LT50 values; 48-h LC50 of $\Delta^{4'}$-dehydrojuvabione, 1.4 mg/liter.*

previously as a reference toxicant for pulpmill effluents (Davis and Hoos 1975), had a 96-h *LC50* of 0.7 mg/liter under the bioassay conditions in this study.

The response of trout to solutions of IJHA differed from their reaction to dehydroabietic acid. In IJHA solutions all fish, including those that survived for 96 h, became lethargic soon after initial exposure and their skin color darkened. By contrast, fish in dehydroabietic acid solutions reacted to stimuli until shortly before death and their skin color did not change. Swim bladders were distended in fish exposed to IJHA or dehydroabietic acid solutions.

$\Delta^{4'}$-Dehydrojuvabione, the most toxic IJHA tested, is present in balsam fir in amounts up to 66% of the neutral petroleum ether-soluble extractives (Manville 1975). This compound was considered to be a good model for $\Delta^{1'}$-dehydrojuvabione, which has been found in mechanical pulping effluents (Leach and Thakore 1974a) and is a constituent of alpine fir (E. P. Swan unpublished results), but was not available in sufficient quantity for bioassay. The increase in toxicity with increasing degree of molecular unsaturation in the series dihydrojuvabione (96-h *LC50, 1.8 mg/liter*), *juvabione (1.5 mg/liter)*, $\Delta^{4'}$-dehydrojuvabione (approximately 0.8 mg/liter) parallels earlier results obtained for the toxicities of long-chain fatty acids to coho salmon, *Oncorhynchus kisutch* (Leach and Thakore 1973). The IJHA were less toxic than resin acids which have 96-h LC50s of 0.2–0.8 mg/liter and are the major toxic factors in mechanical pulping effluents (Leach and Thakore 1974a, 1974b).

IJHA may occur at concentrations up to 5 mg/liter in effluents from mechanical pulp mills operating on mixtures of spruce, pine, and fir. However, these wastes can be detoxified by aerobic fermentation (Leach and Thakore 1974a), and even under bioassay conditions the IJHA were unstable. Thus, provided high concentrations of these compounds do not occur in natural waters due to discharge of untreated effluents or from spraying to control insect pests, it is improbable that they will persist long enough to kill fish. However, the lassitude of trout exposed to IJHA solutions is a warning that sublethal concentrations

could affect the ability of fish to function normally in the environment.

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Functional Hematology of Ringed Seals (*Phoca hispida*) in the Canadian Arctic

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GERACI, J. R., AND T. G. SMITH. 1975. Functional hematology of ringed seals (*Phoca hispida*) in the Canadian arctic. J. Fish. Res. Board Can. 32: 2559–2564.

Hemograms from 20 netted and 6 shot ringed seals (*Phoca hispida*) were studied. Changes in red and white cell values in netted seals offer evidence for functioning sympathetic and adrenal–cortical stress pathways. The very high blood oxygen carrying capacity of ringed seals suggests that the animal is capable of deep or sustained dives. A technique for blood sampling from the vertebral "extradural" vein is described.

GERACI, J. R., AND T. G. SMITH. 1975. Functional hematology of ringed seals (*Phoca hispida*) in the Canadian arctic. J. Fish. Res. Board Can. 32: 2559–2564.

Nous avons étudié les hémogrammes de 20 phoques annelés (*Phoca hispida*) capturés au filet et de 6 tués au fusil. Des comptes différents de globules rouges et de globules blancs chez les

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deux groupes démontrent l'existence de voies métaboliques fonctionnelles de stress sympathique et adréno-cortical. La très grande capacité de transport d'oxygène du sang du phoque annelé suggère que l'animal est capable de plongées profondes ou prolongées. Nous décrivons une méthode d'échantillonnage du sang par prélèvement à même la veine « extradurale », vertébrale.

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THE ringed seal, *Phoca hispida*, is a small circumpolar marine mammal that is distributed abundantly throughout the Canadian arctic. It is the single most important food and fur resource for Inuit (Eskimo) people, and is one of the principal prey species for the arctic fox, *Alopex lagopus*. This seal plays a vital role in the delicate cash economy of the north, but biologically it is poorly understood; much of what is known of its life history has been documented only recently (McLaren 1958; Smith 1973).

So as to appreciate more fully the impact of natural resource exploration in the Canadian arctic, it is necessary to gather more information on the biological demands of arctic dwelling animals. This report is the result of one of a series of studies designed to investigate environmental adaptations in the ringed seal, and to compare the normal animal with those subjected to a number of environmental stresses.

A hematologic study was chosen first because blood can be sampled from wild animals easily and painlessly, yet it yields a considerable amount of basic physiological data. This report deals with observations on the formed elements in blood and their functional significance.

Materials and methods — Twenty ringed seals were netted as part of a tag and recapture operation on Herschel Island, Yukon Territories, during the summer of 1971 (Smith et al. 1973). They were placed in a dry wooden enclosure located within 100 m of the capture site, where they remained until sampled for blood, tagged, and released within 24 h. Blood from the live seals was drawn from the pelvic flipper using the method described by Geraci (1971) or from the extradural intravertebral vein (Harrison and Tomlinson 1956). The latter site is preferable when large quantities of blood are required. The extradural technique, though used by some investigators (Murdaugh et al. 1962; Greenwood et al. 1967), has not been documented clearly. A description follows.

The seal is placed in sternal recumbancy on a smooth flat or slightly convex surface and restrained with "sitting" pressure by a person straddling it, facing the hind or pelvic flippers. If additional restraint is required, another person can manually secure the flippers to the floor or ground. Either a third person or, preferably, the one straddling the seal is now ready to withdraw blood in the following manner. Using firm digital pressure along the dorsal midline of the lower lumbar area, the space between any two vertebral spinous processes can be palpated, (Fig. 1). With the probing finger as a guide, an 18-gauge × 1½-inch (3.8-cm) needle is inserted between the processes, either perpendicular to the long axis of the body, or with the point of the needle angled slightly in a cranial direction. The needle is pushed down into the extradural vein, at which time the pressure may be felt to yield somewhat as the needle enters. If a spinous or articular process of a vertebra is struck, the needle should be "walked" either forward or backward off the bone into the vein. With practice, the technique is simple and yields great quantities of blood. It is less effective than the flipper sampling site when used on intractable animals or on large or obese seals in which case longer needles and more restraint are required.

Samples were also obtained from six seals immediately after they were shot by local hunters. Blood from the dead seals was collected from the heart, brachial arteries, or from the free-flowing head wounds.

The blood for hemograms was placed in tubes containing the disodium salt of ethylene-diamine-tetra-acetic acid (EDTA) as the anticoagulant. Blood smears for differential white cell enumeration, and packed cell volume determinations were made immediately from the EDTA-treated samples. The blood tubes and smears were then stored in a cool area (4–8 C) until they were returned to the laboratory for further processing. The time intervening between sampling and laboratory analyses ranged between 4 and 11 days. Packed cell volumes (PCV) were determined by the capillary-tube microhematocrit method. Total red and white blood cell (RBC, WBC) counts and hemoglobin (Hb) determinations were performed with the aid of a Coulter counter and a Coulter hemoglobinometer, respectively (Coulter Electronics, Hialeah, Florida). Blood smears were stained with Wright-Giemsa stain and 500 cells were tallied for each differential count. Red cell measurements were made with an ocular micrometer. Twenty cells from each seal were measured.

The PCV and Hb were determined in duplicate. The blood for cell counts was divided into two aliquots and each was analyzed in triplicate. Thus, the value for each cell count represents the mean of six determinations.

The age of live animals was estimated by counting the ridges on the claws of the foreflippers (McLaren 1958). Nose-tail length and maximum girth measurements were also taken. All dead animals were aged from the dentinal annuli counted from thin cross sections of the canine teeth (McLaren 1958; Smith 1973).

Results and discussion — The field conditions on Herschel Island were unsuitable for complete

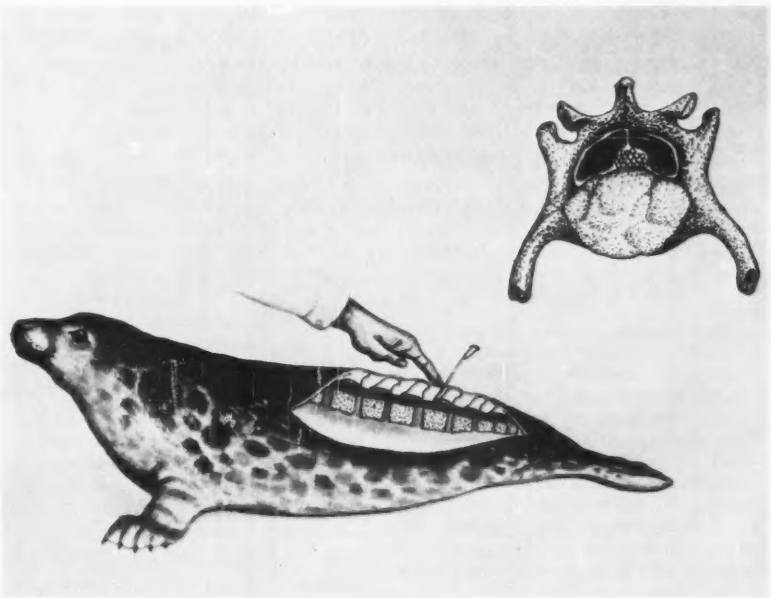

FIG. 1. Semischematic illustration of extradural blood sampling technique. The index finger is used as a guide for needle positioning between the dorsal spinous processes. The insert shows an anterior view of a lumbar vertebra. The blackened area within the arch shows the bilaterally divided extradural vein overlying the spinal cord.

on-site analysis. Therefore, most of the hematologic parameters had to be assessed in a conventional laboratory but only after a considerable time interval. Publication of these findings was postponed until the effects of time and temperature on seal blood could be ascertained. A recent study on the subject has shown that in seal blood stored with EDTA at 4 C, the Hb, RBC, and WBC counts can be assessed accurately for at least 14 days, whereas the PCV determinations should be performed within 24 h (Geraci and Engelhardt 1974). Those conditions were met in the present investigation.

Red blood cells

All of the measured red cell parameters were significantly higher in the netted seals than in the seals that were shot (Table 1). There were no differences in the calculated indices between the two groups. The differences that were noted can be related to stress. Stress by fear, excitement, apprehension, or simply a move to new surroundings, can result in neuro hormonal events leading to contraction of the spleen which forces masses of red cells into the circulation. By this pathway, red cell values can be elevated as much as 25% over resting values in mammals (Schalm 1965). The seals that were shot died instantly; their values may be regarded as normal. Netted seals were trapped and tangled for up to 6 h then moved, restrained, and sampled in a series of events that led to obvious excitement and, judging by the 8–12% increase in red cell mass, a measurable degree of stress. The absence of any differences in the calculated indices between the two groups indicates that the increase in cell mass was due to RBC release only, and not to changes in individual cell volume or cell hemoglobin concentration. That seals have retained this mechanism for a stress-demanded increase in oxygen carrying capacity suggests that it is as useful at sea as it is on land. It might well be adaptive for more sustained diving time, and for greater escape or chase capability.

The total red cell mass of the ringed seal is a reflection of oxygen carrying capacity. As such, it is one of the highest recorded for pinnipeds (Kooyman 1966; Lenfant 1969; Lenfant et al. 1970; Clausen and Ersland 1969). Whereas the elephant seal, (*Mirounga leonina*) achieves a high oxygen capacity using small numbers ($2.54 \times 10^6/\mu l$) of very large ($10.03\ \mu$) cells (Lane et al. 1972), the ringed seal uses the alternate pattern of nearly twice as many smaller ($9.4\ \mu$)

TABLE 1. Red blood cell values in wild ringed seals (*Phoca hispida*) showing capture and age related differences.

Character	PCV (*vol%*)		Hb (*g/100 ml*)		RBC ($\times 10^6/\mu l$)		MCV (*fl*)[a]		MCH (pg)[a]	MCHC (*g/dl*)[a]	cell size (μ)
Regime	Shot	Netted	Shot	Netted	Shot	Netted	Yearling[b]	Adult	Composite	Composite	Composite
Mean	63.0	68.5	24.5	26.5	4.73	5.39	119	135	49.4	38.2	9.4
±SD	6.1	4.7	2.0	1.7	0.42	0.47	9.2	9.3	5.5	1.9	0.26
Low	57	61	21.5	23.0	4.26	4.53	101	116	42.9	36.3	8.0
High	70	75	26.9	29.1	5.46	6.31	153	150	57.4	41.4	11.2
No. of animals	6	20	6	20	6	20	11	15	26	26	26
Level of signif.	$P<0.05$		$P<0.05$		$P<0.01$		$P<0.01$		—	—	—

[a]MCV = mean cell volume (in femtoliters); MCH = mean cell Hb (in picograms); MCHC = mean cell Hb concentration (in grams per deciliter). All indices calculated according to Schalm (1965).

[b]Yearling — young seal, 1 yr old or less.

TABLE 2. White blood cell values in wild ringed seals, showing capture-related differences.

Character[a]	WCB/μl		Band %	Seg. %		Lymph %		EOS %		MON %	BASO %
Regime	Shot	Netted	Composite	Shot	Netted	Shot	Netted	Shot	Netted	Composite	Composite
Mean	7,879	11,472	5.2	27.8	54.2	52.4	30.2	11.6	5.5	2.8	0.8
±SD	3,314	4,911	3.6	15.4	5.3	20.1	6.8	6.9	4.8	1.6	0.4
Low	4,286	6,132	0.4	8.0	46.6	24.6	15.6	3.4	0.6	0.6	0.2
High	13,083	24,774	17.2	50.6	65.2	81.4	38.4	21.8	4.8	7.2	1.8
No. of animals	6	16	26	10	16	10	16	10	16	2.6	25
Level of signif.	not signif.[b]			$P<.001$		$P<.001$		$P<.02$			

[a]WBC = white blood cells; Band = immature neutrophil; Seg. = mature neutrophils; lymph = lymphocytes; EOS = eosinophils; MON = monocytes; BASO = basophils.

[b]Mean of composite WBC count = 10,492 ± SD 4,745.

cells. Both strategies provide equally effective high oxygen capacity (Lenfant 1969). Since this capacity correlates with diving capability in marine mammals (Elsner 1969; Ridgway and Johnston 1966; Kooyman, 1966; Kandinskii 1973), it can be assumed that the ringed seal is adapted to deep or sustained diving.

The MCV in adult seals was higher than in the yearlings, a trend which is consistent with that found in harp seals (Geraci 1971). The significance of this finding is not known.

White blood cells

The white cell counts and distribution are of the same order as have been reported for other pinniped species. However, there were differences in the distribution of cells between the netted and shot seals (Table 2). These differences point to stress with adrenal–cortical involvement. Stress mediated adrenal–cortical secretion produces a triad of elevated neutrophils and depressed eosinophils and lymphocytes (Schalm 1965). This triad was clearly evident in the netted seals. There was a twofold decrease in eosinophils, a 70% decrease in lymphocytes, and a twofold increase in neutrophils which may have influenced the higher total white cell count in the netted group. Schalm (1965) has reviewed this stress-leukocyte relationship which can be reproduced in laboratory subjects by administering or inducing the release of adrenal–cortical hormones. Depression of eosinophils has also been produced by injecting adrenal corticotropic hormone (ACTH) in harp seals, *Phoca groenlandica* (Geraci 1972), offering further evidence that a functional adrenal-stress pathway exists in these species.

Significance

The data on high red cell mass are important in that they provide easily gathered evidence for diving capability of an animal, which in turn helps to put other data on feeding behavior, and habitat selection into some perspective. Such data also helps to narrow down the direction of metabolic or respiratory studies.

The significance of the red and white cell differences between stressed and nonstressed seals can be viewed from a number of standpoints. For practical purposes, they help to give some insight into the physiological status of the animal and, therefore, on its suitability for experiments. Stressed seals respond differently and are less adaptable to captive surroundings (Geraci 1972). As part of this study series, we carried out two identical trials on two groups of ringed seals exposed to an environmental contaminant. One had 100% survival, the other 100% mortality; the only difference was that the latter group had been subjected to an added stress of transportation and captivity (Geraci and Smith unpublished data). Any stress indicators that might help to preview the animal's condition would help to interpret such results.

The difference in blood findings between the groups must be viewed also from the point of view of literature reports on "normal" values in seals. They are alarmingly inconsistent (Lane et al. 1972). Some of the higher red cell values should be reinterpreted in light of capture techniques and the general attitude of the animal at the time of sampling.

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Response of Two Arctic Amphipods, *Gammarus zaddachi* and *Boeckosimus* (*Onisimus*) *affinis*, to Variations in Temperature and Salinity

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BUSDOSH, M., AND R. M. ATLAS. 1975. Response of two arctic amphipods, *Gammarus zaddachi* and *Boeckosimus* (*Onisimus*) *affinis*, to variations in temperature and salinity. J. Fish. Res. Board Can. 32: 2564–2568.

Two arctic amphipods were found to be capable of tolerating a wide range of temperatures and salinities. They were tolerant to both abrupt changes, as would occur in crossing a thermocline, and to gradual changes, as would occur seasonally.

Gammarus zaddachi could survive lower salinities and higher temperatures than *Boeckosimus* (*Onisimus*) *affinis*. Salinity had a statistically significant effect on rates of respiration for both organisms, but only *Gammarus zaddachi* showed significant changes in respiration rate in response to temperature changes. The ecological distribution of these amphipods appears to be in part determined by their ability to tolerate fluctuations in salinity and temperature.

BUSDOSH, M., AND R. M. ATLAS. 1975. Response of two arctic amphipods, *Gammarus zaddachi and Boeckosimus* (*Onisimus*) *affinis*, to variations in temperature and salinity. J. Fish. Res. Board Can. 32: 2564–2568.

Deux amphipodes arctiques s'avèrent capables de tolérer une gamme étendue de températures et de salinités. Ces amphipodes tolèrent des changements brusques, tels que ceux rencontrés lors du passage à travers une thermocline, aussi bien que des changements graduels, tels que les changements saisonniers.

Gammarus zaddachi peut survivre à des salinités plus faibles et des températures plus élevées que *Boeckosimus affinis*. La salinité a un effet significatif sur le taux respiratoire des deux organismes, mais seul *Gammarus zaddachi* montre des changements marqués du taux respiratoire en réponse à des changements de température. La répartition écologique de ces amphipodes semble déterminée en partie par leur aptitude à tolérer des fluctuations de salinité et de température.

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ARCTIC coastal waters are subject to severe seasonal and vertical physical differences. During the winter, water temperatures are below 0 C, whereas in summer they can rise to 15–20 C. Bottom water layers typically can have salinities greater than 60‰ with overlying surface water less than 1‰; these can mix during the summer, but stratification exists most of the year (Atlas and Schofield 1975; Lewellen 1973). Arctic amphipods thus may be exposed in their natural habitat to low temperature (−1.7 C), high salinity (65‰)

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bottom waters, and high temperature (15 C), low salinity (1‰) surface waters; to obtain food they may have to move across the thermocline and experience an abrupt change in both salinity and temperature.

Arctic amphipods have been reported as being euryhaline and temperature tolerant (Holmquist 1965; MacGinitie 1955; Mohr 1953 and Mohr et al. 1961). In the present study, the tolerance of *Gammarus zaddachi* Sexton and *Boeckosimus* (*Onisimus*) *affinis* (Hansen) to both abrupt and gradual salinity and temperature changes was measured. Since environmental stress of two parameters may be synergistic (Kinne 1963; Vernberg and Vernberg 1972), a variety of conditions holding one parameter constant and varying the other, and varying two parameters simultaneously were tested.

Materials and methods — *Gammarus zaddachi* and *Boeckosimus affinis* are two amphipod species that have been found in the Point Barrow, Alaska, region (Barnard 1969; Holmquist 1975; Shoemaker 1955). Considerable confusion exists in the taxonomic literature of the amphipods of this region. *Gammarus zaddachi* has been used synonymously with *G. locusta* Linnaeus, *G. setosa* Dementieva, and *G. oceanicus* Segerstråle (Holmquist 1975). *Boeckosimus affinis* has been used synonymously with *Onisimus botkini* Birula (Shoemaker 1955) and has been confused in the field with *Pseudalibrotus litoralis* (Kröyer).

Amphipods were collected from meromictic Nuwuk Pond at the tip of Point Barrow, and from Elson Lagoon near Eluitkak Pass, Alaska. During June and July 1974 the amphipods were captured both by seining and in wire mesh baskets using whitefish (*Coregonus americanus*) and sardines (*Sardinia pilchardus*) as bait. Salinities and temperatures at the times of collection were measured with a Beckman RS-5 salinometer. In Nuwuk Pond amphipods, mostly *Gamarus zaddachi*, were collected from a depth of approximately 1 m, temperature 8.0 C, salinity 10‰. *Pseudalibrotus litoralis* was also found but in much lower numbers than *G. zaddachi*. In Elson Lagoon *Boeckosimus affinis* was collected from a depth of approximately 6 m, temperature −1.7 C, salinity 27‰. Populations of *B. affinis* were maintained in the laboratory at a temperature of 5.0 C and a salinity of 30‰, and populations of *G. zaddachi* were maintained at 5.0 C and 10‰ salinity. These salinities were similar to conditions at the time of collection for the respective organisms.

To assess the tolerance of amphipods to sudden changes in salinity and/or temperature, animals were transferred in a single step from their maintenance conditions to different salinity and temperature combinations. Twelve salinity levels: 0; 2; 4; 6; 10; 20; 30; 40; 50; 60; 70, and 80‰; and four temperature levels: −2.0; 5.0; 15.0, and 25.0 C were used on these experiments. The various salinities were prepared from a single source of sea water by appropriate dilutions with distilled water, or concentrated by evaporation. Five amphipods in 500 ml water within a plastic container were tested at each temperature and salinity combination. This concentration of amphipods was chosen to avoid overcrowding. Replicate tests were run for each condition. Test amphipods of of various sizes were used in each experiment (range 5–20 mm length). Three signs of life were monitored: locomotion of animals in the container, beating of pleopods, and evasive response to touch. Death of an organism was recorded when life signs ceased. To determine viability of organisms that had been frozen at −2.0 C, life signs were monitored at 0 C after thawing.

To assess the tolerance of amphipods to gradual changes in temperature or salinity, 20 specimens were placed in 1500 ml water at 5.0 C in each replicate plastic container. Salinities were changed 5‰ every 24 h starting from the maintenance salinity. Higher salinities were achieved by the addition of hypersaline water, lower salinities by addition of distilled water. Salinities were raised and lowered until no specimens survived. At 5, 10, 20, 30, 40, and 50‰ salinity, the temperature of replicate specimen containers was raised at the rate of 3.0 C/12 h until no specimens remained alive.

Effects of temperature and salinity on respiration rates were determined using a Gilson differential respirometer within the shock tolerance range of each organism. Respiration rates were determined only within the shock tolerance ranges of the organisms. One amphipod in 5 ml water was placed in each flask. Three-tenths milliliter of 1 N KOH was placed in the well to absorb CO_2 produced. A total of 150 replicate tests was run for each species to establish statistical accuracy. To allow the organisms to acclimate and avoid erratic results as reported by Lockwood (1967), data were collected starting 6 h after a test began. Experiments were terminated 12–24 h later, organisms were removed, rinsed in distilled water, and dried at 110 C for 72 h. Dry weight was determined by weighing with a Cahn electrobalance. Results were recorded as micromole O_2 consumed per gram amphipod dry weight per hour. Data were analyzed by a two-directional analysis of variance.

Results — Tolerance to abrupt temperature and/or salinity changes — At 5.0 and 15.0 C *B. affinis* was able to survive sudden salinity changes from the maintenance condition of 30‰ up to 50‰ and down to 4‰ (Table 1). *Boeckosimus affinis* could tolerate the abrupt temperature change from 5.0 C up to 15.0 C, but not to 25.0 C. *Boeckosimus affinis* could also survive freezing at −2.0 C for 36 h; longer periods of freezing resulted in complete mortality.

At 5.0 and 15.0 *G. zaddachi* could survive abrupt salinity changes from the maintenance 10‰ up to 30‰ and down to 2‰ (Table 1). At 25.0 C the upper limit for survival was also 30‰, but the lower limit was 4‰. *Gammarus zaddachi*

TABLE 1. Tolerance of amphipods to abrupt changes in salinity and temperature. (+ = survival; − = death).

Salinity (‰)	Temp (*C*)					
	5	15	25	5	15	25
	B. affinis			*G. zaddachi*		
0	−	−	−	−	−	−
2	−	−	−	+	+	−
4	+	+	−	+	+	+
10	+	+	−	+	+	+
20	+	+	−	+	+	+
30	+	+	−	+	+	+
40	+	+	−	−	−	−
50	+	+	−	−	−	−
60	−	−	−	−	−	−

could survive sudden temperature changes from 5.0 up to 25.0 C. Thus, *G. zaddachi* could tolerate sudden upward temperature changes of greater magnitude than *B. affinis*. *Gammarus zaddachi* could also tolerate lower salinities but not a sudden decrease in salinity of greater magnitude than could *B. affinis*.

Amphipod size had no relation to the long-term ability to survive temperature and salinity changes. Smaller amphipods (ca 5 mm) did succumb to intolerable conditions more quickly than larger ones (ca 20 mm), but even the largest amphipod could not survive such conditions beyond 24 h.

Tolerance to gradual salinity or temperature changes — When salinities were gradually changed at 5‰ per day at 5.4 C, both species of amphipods were able to survive higher salinities than in the sudden change experiments (Table 2). *Boeckosimus affinis* survived salinities up to 63‰ compared to a maixmum of 50‰ in the sudden change experiments. *Gammarus zaddachi* survived salinities up to 65‰ compared to only 30‰ in the sudden change experiments. *Boeckosimus affinis* survived a gradual salinity dilution down to 4‰, the same lower limit as in the sudden change experiment. *Gammarus zaddachi* survived a gradual dilution to 1‰, compared to a limit of 2‰ when the change was sudden.

Both species were tolerant of higher temperatures when change was a gradual 3.0 C/12 h. At salinities of 5–30‰, *B. affinis* survived temperatures up to 28.0 C, at 40‰ up to 23.0 C, and at 50‰ up to 15.0 C (Table 3). *Gammarus zaddachi* tolerated temperatures up to 33.0 C at salinities of 5–20‰, up to 30.0 C at 30‰, and up to 12.0 C at 40–50‰ (Table 3). Both species were thus able to tolerate higher temperatures at lower salinities.

TABLE 2. Tolerance of amphipods to gradual salinity changes at 5 C.

	B. affinis	*G. zaddachi*
Upper survival salinity	63‰	65‰
Maintenance salinity	30‰	10‰
Lower survival salinity	4‰	1‰

TABLE 3. Tolerance of amphipods to gradual temperature changes at various salinities.

Salinity ‰	Upper survival temp (*C*)	
	B. affinis	*G. zaddachi*
5	28	33
10	28	33
20	28	33
30	28	30
40	23	12
50	15	12

Respiration rates — Respiration rates for both species decreased significantly as salinity increased, indicating lessened metabolic activity (Fig. 1 and 2). The respiration rate of *G. zaddachi* increased as temperature increased, but temperature had no significant effect on the respiration rate of *B. affinis*.

Discussion — This study showed that two species of amphipods, *G. zaddachi* and *B. affinis*, could tolerate a wide range of temperatures and

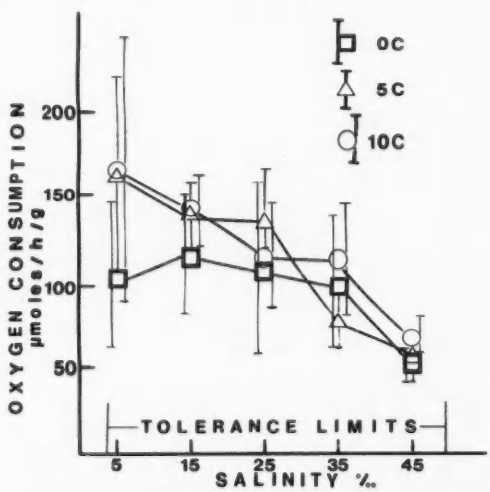

FIG. 1. Rates of oxygen consumption (showing SD) by *Boeckosimus affinis* at various temperatures and salinities.

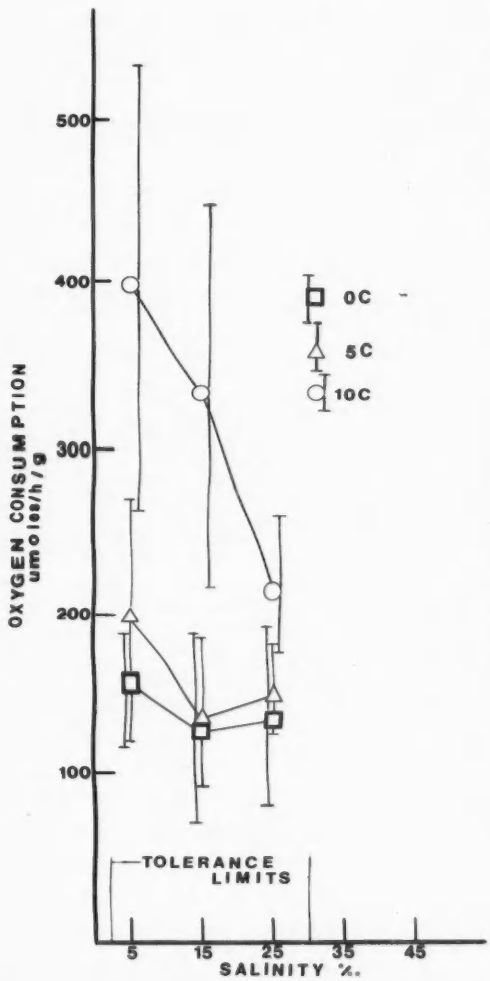

FIG. 2. Rates of oxygen consumption (showing SD) by *Gammarus zaddachi* at various temperatures and salinities.

salinities including abrupt temperature and salinity changes, such as they experience in crossing the thermocline, and the gradual changes associated with the seasons.

Unlike the extensively studied *G. duebeni* Lillj, found in temperate zones, which can tolerate supranormal temperatures better at higher salinities (Kinne 1970), both *G. zaddachi* and *B. affinis* had lower upper lethal temperatures at higher salinities. Again, unlike adult *G. duebeni* which can survive fresh water indefinitely (Kinne 1964), *G. zaddachi* could tolerate only 1‰ as the lower salinity limit.

Respiration rate is a sensitive measure of physiological state and was used to monitor the effects of sublethal temperature and salinity change on amphipods, and as an indication of their ability to adapt to environmental change. As with other euryhaline organisms, including *G. duebeni* and *G. locusta* (Kinne 1964; Prosser 1973), the rates of O_2 consumption for *G. zaddachi* and *B. affinis* increased at lower salinities. Increased respiration at low salinities may have been associated with increased ion regulation. At high temperatures increased respiration may indicate elevated enzyme activity.

Nuwuk Pond experiences higher temperatures in the summer and lower salinities in surface waters than the much larger Elson Lagoon. *G. zaddachi*, better able to tolerate low salinity and higher temperature conditions than *B. affinis*, was the dominant amphipod in Nuwuk Pond. In the more stable Elson Lagoon *B. affinis* was the dominant amphipod. Our experiments showed that both organisms could survive in the lagoon and, indeed some *G. zaddachi* was found there. One should note that during severe storms there is mixing between Nuwuk Pond, Elson Lagoon, and the Arctic Ocean, allowing for movement of organisms from one to the other (Mohr 1953).

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PERSPECTIVES

International Joint Ventures in Fishing and 200-Mile Economic Zones

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TOMLINSON, J. W. C., AND I. VERTINSKY. 1975. International joint ventures in fishing and 200-mile economic zones. J. Fish. Res. Board Can. 32: 2569–2579.

This paper examines the impact of various strategies that may be adopted by coastal nations for utilization of fishery resources assigned to their jurisdiction under a system of 200-mile economic zones, such as that apparently favored at the Caracas Conference on the Law of the Sea. Benefits and problems that are likely to arise out of alternative approaches are discussed in the context of experiences of developing countries with foreign investment in other sectors. The paper suggests that various forms of joint ventures appear to have the greatest chance of success in achieving both effective utilization of resources and beneficial development of the coastal nations.

TOMLINSON, J. W. C., AND I. VERTINSKY. 1975. International joint ventures in fishing and 200-mile economic zones. J. Fish. Res. Board Can. 32: 2569–2579.

Les auteurs examinent dans cet article les répercussions des diverses stratégies qui peuvent être adoptées par les nations côtières en rapport avec l'utilisation des ressources halieutiques tombant sous leur juridiction avec un système de zones économiques de 200 milles, auquel on a semblé accorder une préférence à la conférence de Caracas sur le droit de la mer. Ils analysent les avantages et les problèmes susceptibles de découler de diverses méthodes d'approche, dans le contexte d'expériences des pays en voie de développement avec des investissements étrangers dans d'autres secteurs. Les entreprises conjointes, sous diverses formes, semblent avoir le plus de chances de succès à réaliser à la fois une utilisation efficace des ressources et un développement profitable des nations côtières.

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International Joint Ventures in Fishing and the 200-Mile Economic Zones

ONE of the most interesting subordinate themes that has entered into discussions of future utilization of the world's fishing resources since the 1974 Caracas conference on the Law of the Sea is that of joint ventures. This interest has been enhanced by virtue of the fact that the most significant single result of the conference now appears to be a consensus in favor of clear demarcation of zones of interest and economic authority – more specifically the 200-mile limits. In fact a large majority of the 148 members of the conference favored a 200-mile zone of some kind, from one in which the coastal state had prior or preferential interest (Nigeria, L21; Landlocked Bloc, L39), through complete power over economic issues (EEC, L40), to complete economic and political authority – a territorial sea concept (Brazil; African States, L82). Adoption of such demarcation would mean that sovereignty over a large proportion of currently exploited fishing resources is definitely established. Under such a system, the legal, or at least the morally accepted basis for control of resources is clear and with it the basis for allocation of potential exploitative capability. Allocation will be the prerogative of the nation within those limits the location of harvesting occurs.

Given the availability within these nations of fishing capacity sufficient to harvest these new national resources at reasonably sustainable levels, one might expect that they would themselves undertake full exploitation and export any sur-

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plus above domestic requirements. For some of the developed nations this will be the case and ultimately it may be the universal situation.

> "The long-term is for Canadians. Canada is not only going to reach out and encompass all of the living resources of her continental shelf and slope, we are going to make sure that they are harvested by Canadians, in Canadian-owned vessels, and processed in Canada as well" (Jack Davis, Canadian Minister of Environment, March 1974).

In the meantime, however, many of the coastal nations with newly endowed fish resources will be unable to exploit them because of inadequate fishing and processing capabilities or technological potential. In a protein-deficient world, with food prices escalating due to imperfections in supply functions and production costs, these resources would find ready markets and lack of harvesting would then represent revenues foregone by the owner states.

Alternative Strategic Approaches

The problem of matching technological, managerial, and financial capabilities with potential domestic abundance of raw materials is hardly new. This dilemma is one that has been attacked by many underdeveloped nations host to oil, mineral, food and forest product resources, with varying methods and varying levels of success. Descriptions of the findings from research and experience in the fields of foreign investment, trade, and development of these industries (Robock and Simmonds 1973) recognize a range of strategic alternatives that are also applicable to the changed ownership pattern of international fishery resources.

Possible strategic approaches can be classified into five major types:

1) The *Null Strategy*, is to do nothing, at least in the short-term;
2) The *Independent Strategy*, would be to try to go it alone;
3) A *Licensing Strategy*, charging foreigners for the right to fish;
4) To encourage foreign direct investment in fishing via a *Foreign Subsidiaries Strategy*;
5) To insist that such investment participate in a *Joint Venture Strategy*.

This classification has to be elaborated and modified for consideration of strategic alternatives from the point of view of the main protagonists, host nations and foreign investors. In some cases there are significant variations between different versions of an approach. In others the impacts of different strategies may be similar enough for them to be combined. Thus joint operations between foreign partners may in effect be merely a subset of the *Foreign Subsidiaries Strategy* for the host country, while intergovernmental joint ventures vary significantly from those between private sector interests or mixed forms (Kapoor and Robinson 1971). The strategies also vary in their reversibility and sequentiality over time, the level of disruption caused by change or transition to an alternative approach.

Null Strategy

In any decision situation there is always the choice of doing nothing. Usually this implies short-term opportunities foregone, but may also avoid longer term costs of alternative courses of action. Faced with an unfavorable set of such alternatives, it can be seen as a minimax or Wald criterion solution which attempts to minimize the magnitude of undesirable outcomes (Kaufmann 1968). Theoretically, the desirability of such a strategy could be estimated in terms of the assets at stake (Pratt 1964), or the costs avoided and benefits foregone by the host country (Little and Mirrlees 1969). In practice the uncertainty and complexity of future data and outcomes in the context of national development make accurate calculation impossible (Wellisz 1971).

Given the absence of reliable information to predict the future, an attractive alternative is to avoid making a decision for as long as possible (Ansoff 1965). In this sense the *Null Strategy* is one of deferral. In the general case of nations owning natural resources, its adoption may prevent their depletion by means that only generate limited or unsatisfactory benefits for the owners, at a cost of delaying possible national development stimulated by exploitation of such resources. Since fish constitute a renewable resource, this strategy would appear to have less relevance for the developing coastal states, subject to two reservations.

The first is concerned with the assumption that biological criteria for effective maintenance of "maximum" or "optimum sustainable yield" or any other acceptable fishery management goal are available and can be implemented. With the exception of a very few species such as cod, haddock, halibut, some salmon, etc. such an assumption is open to considerable doubt (Gulland and Robinson 1973; Larkin 1972). Even if the arguments could be resolved into some reasonably accurate consensus, there still remain even stronger doubts concerning the capability to manage fishery resources effectively: "performance in management of coastal fisheries under national

jurisdiction has not been impressive. The states concerned have not even succeeded in maintaining equilibrium yields of endemic resources, unavailable to foreign fishermen" (McHugh 1974).

A second depends upon the nature of the final definition of sovereignty over coastal waters. If this results in economic zones or territorial waters of one form or another, then some coastal states may be able to accept the short-run costs and risk deferring their strategic decision regarding development of fishery resources. If, however, the result is some form of conditional or limited preferential rights, the *Null Strategy* would involve the risk that the resources would be preempted in the short-term by fishing interests with capability for immediate exploitation. This risk is compounded by the doctrine of "established custom and practice," the danger of creating long-term precedents which would inhibit later utilization of the resources by the coastal nations themselves. "South Africa (for example) may decide that the risk to her fisheries is too immediate for her to be able to afford to wait" (Fishing News International October 974).

Strategy of Independence

In view of the political aspirations for independence on the part of developing nation states, coupled with ambitions of achieving economic sovereignty which will yield maximum domestic benefits from national resources (Fayerweather 1969), the strategy that is intrinsically and emotionally most appealing may well be to try to go it alone (Stikker 1968). The argument is strengthened by a general mistrust of the intentions of the developed countries and fears of economic imperialism.

Whatever its attractions, however, this strategy would appear to be impossible, almost by definition. If the less-developed nations had the technological capability and human resource skills necessary for independent development, it seems contradictory that they would have remained less-developed, unless one assumes a conspiracy exists in the system of international trade (Prebisch 1963). The net effects of colonial domination may have created elements similar to those of a conspiracy, but even when these are allowed for, the only cases of successful strategies of independent development in the 20th century are those of Japan and the USSR. Those successes depended upon supportive sociocultural values and homogeneity, plus their integration with the economic and political systems, or upon a completely dominant political hegemony in the country. These created a basic capability and its concentrated application which do not appear to be replicated in any of the developing nations. For the coastal nations, therefore, this strategy for utilizing their fishery resources is probably not valid until a later stage in their own development.

Licensing Strategy

A solution that may be viable with a relatively limited administrative, legal, and economic infrastructure would be to license exploitation by foreign fleets, recouping part of the lost revenues in the form of license fees, royalties, or even export taxes. The main characteristics of this solution would be its short-term expediency and benefits. It would create revenues for the owner nation immediately, but these would be only a limited proportion of the potential represented by the resource licensed. It would do little for the development of national capacity such as that necessary for domestic harvesting and utilization of the fish resources in the future (Drucker 1974). Without access to the necessary technology, the capital created by license revenues would be limited in its substitutability into local fishery capacity (Eckaus 1973). Without training and experience in fishing, the necessary local skilled and managerial manpower would not develop.

Like the *Null Strategy*, this approach is likely to have limited impact on national development, especially for those coastal nations that have little capability of policing their resources against unauthorized utilization by foreigners. Both should probably be considered as short-term reactions or even holding decisions rather than viable long-term strategies. A *Licensing Strategy* appears to have certain advantages over doing nothing. Revenues obtained will at least have beneficial balance of payments effects and may raise income levels initially. Given that the terms and duration of licenses are clearly drafted and applied, there is less danger of preemption of fish resources, or the creation of "traditional" precedents for fishing rights of foreign interests in coastal states' new domestic waters.

The tone of the proposals and debate at Caracas and the basic attraction of the financial return, "no state would turn back the revenue to be got from licensing fees" (Johnson 1974), suggest that this alternative will be the immediate strategic choice of coastal states, certainly for allocation of fish surplus to their own harvesting capacity. Its possible danger to their long-term interests lies in the very simplicity and ease with which national revenues can be increased. This may well create inertial effects when compared

with the costs and disruption perceived in transition to a strategy likely to create more synergy for national developement. If this is the case, it will at least delay, if not inhibit, such transition. There is also an associated danger of exhausting the resource, "As one African delegate observed, the possibility of granting licenses to third states made it more likely that overutilisation would occur than underutilisation" (Johnson 1974).

Foreign Subsidiaries Strategy

This alternative would suggest that fish-owning nations should encourage the establishment of foreign-owned harvesting and processing companies within their national jurisdiction. In terms of control over utilization of the resources, this would be an improvement over simple licensing. It would also generate a greater local share of revenues. Not only could taxes on earnings of the foreign subsidiaries be added to those accruing from licenses to operate, but revenues would also be generated from some increase in local employment. Sales of product by the subsidiaries would now generate foreign exchange from exports since title to the produce would have to pass through host country jurisdiction (Strikker 1968). If local employment could be suitably encouraged this would also help to develop a pool of trained and experienced manpower. Finally, host countries could also demand disclosure of certain information on location of resources, management, and research development results.

In spite of these benefits, the experience of host nations with resource extractive foreign direct investment indicate that this alternative has important limitations, certainly in the medium to long term if initial conditions do not change (Litvak and Maule 1970). The main problems are possible enclave development and limitations to the local share in values added and earnings from the resource.

Creation of an enclave leads to duality in the local economic and social system which reduces the potential benefits to national development from diffusion of foreign technology (Levin 1960). This has been a problem in the past with some foreign investment in the plantation and mining sectors (Myint 1965). It would be less of a problem to the extent that fish produced was sold for domestic consumption. Since by definition in many cases the resource would be exploited primarily for export markets, this is a limited safeguard. The problem can theoretically be alleviated by government intervention to stimulate diffusion and transfer of benefits. "Argentina will not bar foreign participation in the fishery, but any outsider wanting to work inside the limits will be confined to supplying fish to local processors, or his own domestic market, and will be expected to invest in shore plant in the south" (Fishing News International May 1974). In spite of the increasingly recognized powers of sovereign governments in the modern world and their improved understanding of the art of economic governance, effective intervention is a function of the capability for positive economic control and this is low in most governments of developing nations.

Limitation of the local share in earnings and benefits from the operations of foreign subsidiaries is partly a pro rata argument, in that lack of ownership clearly limits participation in earnings from the business. More importantly it also implies lack of participation in determining policies in favor of local national interests. Ostensibly this would be overcome by the powers of a sovereign host government, but experience suggests that effectiveness of such intervenion is limited without access to genuine operating data, which is in turn limited to direct participants or owners. "Under these circumstances, the joint venture is an instrument of the host government for increasing its control" (Mikesell 1970).

Even though the route of utilizing domestic fishery resources by encouraging the establishment of foreign subsidiaries has its weaknesses, it may well be a viable short-term component of a longer term strategy, except in special cases where public opinion, particularly in developed countries, may preclude it, i.e. Alaskan antipathy towards Japanese long-distance fleets. The situation with respect to foreign direct investment is one of bilateral monopoly. Initially the owner government has the resources, while the foreign fishing company or agency has the capability and the technology. In foreign investment theory (Hymer 1960), the initial monopolistic advantage usually lies with the owner of the technology who can impose conditions to his own choosing at this stage.

Once the investment is made and as the resulting technology is diffused through the local economic, social, and political systems, the relative advantage shifts towards the host country. It is now in a position to unilaterally change the conditions of the game, governed only by possible opportunity costs of discouraging other foreign investors, its ability to effectively reproduce the efficiency of the operation, and the impact of possible loss of potential future inputs of advanced technology by the original foreign investor (Vernon 1971). As these changes develop, the logic of the situation leads to other strategies such as the creation of joint ventures as an ac-

TABLE 1. Japanese Joint Ventures in Fisheries as of March 1972 (Honda 1973).

	Latin America	Asia and Oceania	Africa	Europe	North America	Totals
Shrimp trawling	2	14	5	—	—	21
Other trawl fishing	3	3	1	—	—	7
Skipjack Tuna fishing	1	3	1	1	—	6
Other fishing	4	—	1	—	2	7
Subtotal fishing	10	20	8	1	2	41
Aquaculture	—	7	—	—	1	8
Cold storage	2	8	4	—	—	14
Fish processing	2	2	1	1	6	12
Others	1	—	—	—	1	2
Total joint ventures	15	37	13	2	10	77
Capital investment ($'000)	9,458	16,641	4,296	2,559	4,099	37,053
Japanese investment "	7,277	12,163	2,251	984	2,381	25,056

commodation which, potentially at least, permits both parties to continue to benefit.

The Developing Environment for Joint Ventures

It may be that in view of the strength of demand for fishery products in world markets, the new owner nations can bargain for better initial conditions than the standard bilateral monopoly model would suggest. This argument would be enhanced by the fact that many of the major interests in international fisheries are state agencies whose ideological and political preferences are likely to be for cooperative arrangements, rather than for situations that could be attacked as unilateral dominance or economic imperialism. The positions of the USSR and other communist nations are, for example, becoming more flexible with respect to joint operations with noncommunist interests:

> "It is highly likely in years to come that development of the world economy will continue to be marked by a tendency ... which will, without doubt, make international cordination of production and research programmes of nations and individual enterprises increasingly beneficial" (World Economics and International Relations, Moscow 1973).
>
> "We have also decided to resort, in relations of international economic cooperation to the organization of joint companies, both in Rumania and in other countries. We have set out to do this bearing in mind the new development conditions of the world economy and the need for new forms of economic relations, namely direct cooperation in production. This allows for a broader expansion and more enduring economic relations with other states, and at the same time, the possibility of introducing advances of contemporary science and technology into production more rapidly" (Ceausescu 1973).

This reorientation is shown more specifically in the creation in several communist states of legislation to establish domestic conditions for joint activities with Western interests (McMillan and St. Charles 1974). These ground rules, with their emphasis on concern for protection of local interests, give an indication of the type of relationship the agencies of these nations will look for in their own investment abroad in industries such as fishing. Moreover, the monopolistic centralized interests of socialist countries are becoming increasingly interested in investment abroad, even in international production (Vernon 1974).

Whatever the bargaining or ideological position, however, sooner or later the development needs of many of the coastal states seem likely to lead to the creation of joint ventures with foreign interests to utilize their fishery resources in their own best national interests. This process was already under way before the redefinition of 200-mile limits. It had been anticipated by FAO with the creation of a service to include joint ventures as one of the two main arms of its reorganized Fishery Industries Division in 1973. In the same year the government and fishing companies in Japan set up the Overseas Fisheries Cooperation Foundation to advise and assist in the development of joint ventures. There was considerable evidence in the Caracas debates that less-developed states, in general, showed a preference for various forms of joint venture arrangements.

Even an incomplete scanning of the news reports on world fishing topics comes up with more than 80 new joint ventures in various fisheries in the last four years, a large proportion of them involving Japanese collaboration with African or Pacific national interests (Table 1).

Like most Japanese investments abroad to date (U.N. Transfer of Technology 1971), these ven-

tures were set up in order to secure foreign sources of materials (Duval 1974), in this case food, for the Japanese economy. These criteria are consistent with the models of foreign investment decisions (Aharoni 1966), including those in extractive industries (Behrman 1969), and it is reasonable to predict that most joint ventures in fisheries will be set up as a replacement for some of the current long-distance fishing operations of foreign parent interests for their domestic markets.

Similarly, this prediction would suggest that in the early stages, few joint ventures will be established to serve third markets. Apart from Norway, Iceland, Denmark, and the USA, or for cases of servicing specialized or luxury markets, there are few potential foreign interests of adequate technical competence whose primary concern is likely to be with third markets. Eventually, the separate interests of the joint ventures themselves, coupled with alterations in international patterns of supply, may well lead to greater sales to third markets. Initially, however, this is unlikely to be an important determinant for the creation of joint ventures.

The Joint Venture Strategy

If the promotion of joint ventures is likely to become a dominant strategy for exploiting newly defined fishery resources, it is worth looking in detail at some of the strengths and weaknesses of this type of structure. In view of the data and information available concerning international business joint ventures with the extractive and manufacturing industries (Mikesell 1970), these seem to provide a useful model for considering similar ventures in fisheries.

In operational terms a joint venture is the commitment for more than a very short duration, of funds, facilities, and services by two or more legally separate interests, to an enterprise for their mutual benefit (Robinson 1964; Friedmann and Kalmanoff 1961).

A preliminary typology can be established in terms of one of the main structural characteristics, the identity of the major participants (Tomlinson 1970). The types of venture include *National Joint Ventures* between two or more interests from the same foreign country and *Foreign International Joint Ventures* with partners of different nationalities, excluding that of the host nation. Neither of these is likely to suit the aspirations of coastal states. Both can in fact be considered more representatively as part of the *Foreign Subsidiaries Strategy* from the point of view of host countries, since the jointness in decision-making occurs abroad. *International Joint Ventures*, in which some part of the commitment is by local nongovernmental interests, will be feasible in states with an embryo fishery industry already in existence, or in which there is a viable private sector including potential investors in new fishing operations. This would probably be the format most attractive to West European, U.S., Canadian, and Japanese firms in the industry.

Most popular with the developing coastal states is likely to be some form of joint operation where at least part of the participation is by the host government, while another is by foreign technology contributors. The latter may be private interests in *Mixed International Joint Ventures*, an agency of one or more foreign governments in an *Inter-Governmental Joint Venture*, or even a multilateral agency acting as a partner in a *Multilateral Joint Venture*. Of these forms, the first is already a feature of international business in the extractive industries, while the second is the vehicle preferred by socialist states. The last may be an attractive concept, but appears to be an unrealistic alternative because of limitations in the existing terms of reference and resources of most multilateral agencies.

This basic typology can readily be extended and refined further by distinguishing various shares in ownership, distinguishing for private sector partners in particular, between majority, minority, and 50–50 participations, with their associated implications for financial control. In political and economic terms elaboration could incorporate special relationships and complementarities to assist in defining probabilities of success, or even of the establishment of specific joint venture relationships. Another important distinguishing variable would be the relative contribution of enabling resources by the partners. Such a typology could well form the basis for any attempt to establish advisory guidelines by FAO, at international assemblies such as the Geneva Conference, or elsewhere.

With the exception noted above of purely foreign-owned joint ventures, the general format has legitimacy with respect to the political and development ambitions of the prospective host nations (Chenery and Taylor 1968). This is at least partly due to the psychological impact of the jointness of the endeavor and the positive effect associated with the implicit idea of cooperation. More specifically, the potential threat to sovereign rights and capabilities of host states is perceived as reduced by the participation of local interests (UNCTAD Trade Expansion 1966). Legitimacy for development is both direct and indirect. In the first round there is development of a national resource; through multiplier effects and linkages, this diffuses through the socioeconomic systems

of the host country, generating further revenues, investment, employment, and opportunities.

Because of the inputs of resources, technology, and access to markets by the foreign partner, the joint operation has a high probability of achieving economic viability. This too creates direct and indirect benefits. As a viable concern, not only are revenues generated, but the costs of developing the fishery resources are more than covered. This avoids the need for development subsidies, or at least reduces the subsidy impact on national accounts to a level appropriate to support of an "infant industry" in the early stages of a new venture (Kindleberger 1973). Indirectly, a viable operation creates externalities favorable to related and dependent activities — lower cost, reliable sources of supply for processing plants — new and larger potential markets for local producers of nets, gear, materials (Hirschman 1958).

These benefits are theoretically not peculiar to a joint venture as compared with fully foreign-owned subsidiaries. In practice, however, the latter have a predilection for the use of existing, related, or familiar sources of supply and market relationships which is difficult to legislate against effectively. Jointness of participation in decision making provides a more effective basis for direction of procurement and marketing policies and for scrutiny of compliance (U.N., Impact of Multinational Corporations 1974).

In terms of the development of human resources, foreign investors can usually be induced to create a certain amount of employment and the associated training of local nations. The main bulk of the employment, however, tends to be in unskilled and semiskilled grades and the training limited to semi-skilled and supervisory levels (I.L.O. 1973). Technical and managerial activities remain the prerogative of foreign personnel as long as possible. In the early stages of a new fishery operation in a less-developed nation, this would of course be a necessary condition under any circumstances.

The advantages of a joint venture for the host country in this context lie in the speed at which managerial and technical personnel are likely to be developed and to become experienced in the new industry (Drucker 1974). Even if a local partner does not have people of sufficient potential caliber immediately available for retraining, it is very hard for the foreign interest to resist a partner's insistence that they should provide effective local managerial inputs as soon as possible, throwing the onus for ensuring effectiveness onto the foreigner. The faster this process, the sooner is created a cadre of technically trained managers available to the host economy — either through "pirating" by other local firms, or as embryo entrepreneurs on their own account (Glade 1967).

Among the indirect off-shoots of this particular contribution is the more rapid creation of two other potential capabilities. First, without effective locally based fishery operations, the only opportunity for researchers, technicians, and even administrators to leaven theoretical work with practical experience is through secondment to a tolerant foreign interest or institution (Wilson 1968). The latter are not always accommodating, because of perceived conflicts of interest, or their accommodation may be limited. Whether the role is specifically induced through legislated incentives, or is merely an intrinsic feature of their existence, new local fishery ventures are likely to provide potential workshops for the development of a national technical capability, including "the first and most important technology, the technology of informed choice" (U.N., Impact of MNCs 1974). More specifically, they can constitute a particularly viable nucleus for effective utilization of national funds and resources for generation of a domestic capability in research (Pavitt 1971). Jointness of the venture would encourage the consideration of host nation interests and remove some of the inhibitions of governments in allocating funds for research, since at least they would be going to domestically shared concerns (Bourgault 1972).

A second off-shoot concerns control over any new zones of influence. One of the problems in policing the new coastal limits for nations without a naval tradition will be the lack of effective domestic deterrent ability. This means basically two things: first, control of sufficient force in military terms to exercise sovereign rights against interlopers; second, access to adequate information to apply such force in time for it to take effect. Although the issues were not resolved at Caracas (Johnson 1974), it is likely that this will prove an early concern and that creation of such a force will receive a high priority from the governments of coastal states. Growth of a fishing fleet leads to growth of a merchant marine capability, which is in turn a necessary resource for an operational naval force. The presence of domestic vessels fishing for the same resources increases the number of data points available to the national scanning mechanisms. These processes too will be enhanced by local participation in fishery joint ventures.

So far the discussion has concentrated on positive aspects of joint ventures. The picture is not always clearly defined or rose-tinted. Horror stories of joint ventures that have gone wrong are legion in the literature of business folk-lore. There

are always potential conflicts of interests between partners and recriminations when these are not foreseen, or coping mechanisms established as part of the relationship (Behrman 1974). There will inevitably be problems in joint fishery activities similar to those experienced in other industries. In fact these have already arisen; the Japan Fisheries Association noted in 1973 that many of the joint ventures begun since 1954 had closed down. "Reasons for the failures are given as inadequacy of pre-investment investigation, failure in business management, changes in political and economic situations and various regulations governing foreign investment in the host countries" (Fishing News International May 1974). However, in none of these cases do the dangers preclude the basic viability of the format for the type of situations involved.

The most vociferous opposition to joint ventures has always come from spokesmen for internationally operating companies. As a general rule, the less the firm's actual experience with this type of operation, the louder their condemnation. Executives with direct experience of joint ventures certainly recognize the negative possibilities and have often in fact been involved in them (Mikesell 1970; Bivens and Lovell 1966). Nonetheless, they also recognize potential mutual benefits and the possibility of avoiding or resolving conflicts. "I think that the promotion of foreign investments in the developing countries may depend more and more upon the acceptance, whatever the problems, of partnership with local interests. In the beginning, because of the general lack of any local private capital, this may mean the acceptance of participation by government in the equity" (Loudon 1965).

Objective analysis of the difficulties for a firm that can arise from this type of relationship suggets that there are only two real negative aspects. The first is again the pro rata argument, this time reversed. If the rate of return from an investment in a fishery venture is high, then, other things being equal, sharing the investment implies a reduction in the absolute volume of these attractive earnings. If the firm has adequate capital and resources to undertake the whole investment on its own and the opportunity costs of foregoing other alternatives are low, it may naturally be reluctant to share.

The second is a question of increasing operational efficiency through independence in decision making. In the early stages especially, fragmentation of decision responsibility and the need for resolving conflicts of interest can slow down the speed with which start up, or the achievement of efficient on-going operations can be attained (Kahn 1965). In these initial stages of technology transfer, the contributions of local associates may well be of little importance. A subset of this argument concerns the limitations upon the freedom of a firm to allocate transfer prices so as to maximize the global return to its overall operations in cases where it has to consider the interests of local partners. In practice, faced with the increasingly suspicious scrutiny of shadow prices by sovereign host governments, this freedom has been increasingly curtailed, even for multinational networks of fully owned subsidiaries (Arpan 1972).

Other arguments are essentially subjective, or are concerned with issues that basically involve interference with the freedom of investors to operate as they find most suitable.

Whether objective or subjective, however, in any event the validity of such objections is constrained by two criteria. If the opportunity is attractive or important enough to outweigh negative aspects, it is likely to be undertaken in any case. If, moreover, a joint venture is the method of operation legally prescribed by the owner of the resources, the objections are virtually irrelevant and what matters is minimizing the potential negative impacts. Where the resource in question is a source of protein that is vitally important to the foreign investor's own parent country markets, therefore, and alternative sources are limited, the opportunity is probably too important to forego. When the name of the host nation's game is joint ventures, there is little choice. This appears likely to be the decision situation for foreign fishing interests which will be created by the coastal states.

The question of anadromous and deep-sea fishery resources was left hanging after Caracas and remains unresolved after the Geneva LOS III Conference in 1975. Part II of the Informal Single Negotiating Text produced by the Second Committee at Geneva starts to deal with the issue. Article 54.1 recommends that "Coastal States in whose rivers anadromous stocks originate shall have the primary interest in and responsibility for such stocks." Article 54.2 assigns to Coastal States regulation, conservation, and determination of allowable catches. Then, however, the nullifying provisos take over. The State of Origin must "minimize economic dislocation in other States fishing these stocks" (Article 54.3[b]); "enforcement of regulations regarding anadromous stocks beyond the exclusive economic zone shall be by agreement between the State of origin and other States concerned" (Article 54.3[d]); "The State of Origin and Other States shall make arrangements for the implementation

of this article, where appropriate, through regional organizations" (Article 54.5), (U.N. 1975 — hardly a clear and directive set of recommendations.

There are a number of reasons for the lack of definition in this area. An element of euphoria over the newly acquired resources of their increased territorial limits may have reduced the zeal for immediate negotiations over wider areas by the coastal states. For their part and in the absence of a mutually beneficial, clearly defined solution, the long-distance fishing nations probably prefer to continue operating under existing conditions which give them a competitive advantage in exploiting their technology — "the big powers see the conference as a Third World attack on the Status Quo — it is in their interest to block progress in areas where they feel disadvantaged" (Gold 1974). Once the location of a problem shifts from clearly recognizable and accepted areas of definition, such as continental shelves or n-mile limits, to the common domain, it becomes in any case much more difficult to resolve.

Resolution requires a higher level of international cooperation and effective multiple participation. At the moment, the prime movers in bodies regulating regional and international fisheries such as NEAFC, ICNAF, ICSEAF or the tuna, halibut, or whaling commissions are usually the developed nations. Developing coastal states have limited interests in deep-sea issues and even less ability to participate in regulation themselves. There is also considerable mutual suspicion of each other's interests and intentions between the two groups.

A major theme throughout this paper has been the potential of joint activities to speed up the development of the fishery capabilities of the less-developed coastal states. This would clearly include both the interest and the ability to take an effective part in deep-sea regulation. Participation in a domestic fishery industry and the experience gained from exploiting resources within broad national territorial limits will undoubtedly increase the enthusiasm of the new coastal fishing states for sharing in the harvesting and utilization of deep-sea resources. In terms of practical contributions, they would then be likely to have operationally valuable facilities to offer. Even more important, given their development of viable research capabilities, would be the possible contribution to scientific rather than simply emotional debate on methods of international control (Knauss 1974).

There is, moreover, a further encouraging potentiality. Studies of industrial joint ventures indicate that successful collaboration breeds a favorable attitude towards further joint operations; "Although many companies have looked at the advantages and disadvantages of joint ventures and have decided to invest only where complete control is possible, others have had long and agreeable experiences with both majority control and minority interest ventures. With the continuing increase in nationalism and the growing demand for private capital around the world, many believe that the international joint-business venture will become the most important vehicle of private foreign investment" (Bivens and Lovell 1966).

The power of this effect is underlined by some of the strictures of the U.S. antitrust rulings against joint activities of American firms abroad. "The intimate association of the principal American producers in day-to-day manufacturing operations, their exchange of patent licenses and industrial know-how, and their common experience in marketing and fixing prices may inevitably reduce their zeal for competition *inter sese* in the American market" (Wyzanski 1950).

To the extent that such attitudes are enhanced by the growth of joint fishery ventures between interests from long-distance fishing developed nations and less-developed coastal states, later cooperation in the regulation of deep-sea resources is likely to be more feasible and less controversial.

A major determinant of the degree of success in any joint venture is likely to be the thoroughness of preliminary forecasting of situations in which conflicts between partners can arise. This needs to be combined with prior negotiation of solutions, or the basis for resolution, coupled with careful drafting of the terms and conditions of relationships (U.N., Impact of MNCs 1974). The effectiveness of this vital part of the process demands knowledge and experience that are unlikely to be among the resources of the principal actors in fishery joint ventures in the near future. For these reasons, one of the most urgent international fisheries study group activities prior to Geneva and preferably independent of it, except for generating consensus and support, should be concerned with creating practical guidelines for setting up and operating international joint ventures in the fishing industry.

As a final note, although the topic here has been joint ventures in fishing, the problems that will arise in exploiting sea-bed mineral resources in the new economic zones will be similar in nature. Because the technology involved is less established, solution of the problems may be less urgent in the latter case. The initial tendency towards joint ventures may also be weakened because of the greater bilateral monopolistic bargaining power of the foreign interests created by the relatively complex and unique technology. In this

situation, however, there is clearly a need for the preparation of suitable guidelines for strategic decision making and this process would benefit considerably from the development of relevant precedents, procedures and experience in the case of fisheries management in these same zones.

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BOOK REVIEWS/COMPTES RENDUS

Experimental Marine Biology. *Richard M. Mariscal [ed.] Academic Press, Inc., Publishers, New York, N.Y., 1974. x + 373 p. $18.50.*

Dr Mariscal points to the increasing interest in marine organisms as the reason for bringing together some of the recent work in the various experimental marine biological fields for the benefit of interested students and experienced researchers. This eight-chapter volume covers all aspects of biological organization from molecules to communities, plus an article on the ABC's of closed system aquariums.

The diversity of the book is such that it would be difficult for any one reviewer to have expert knowledge in all of the fields. Thus, I have read the book from the point of view of one of its objectives: "to gain insight into the problems and perspectives of each discipline."

Connell's chapter on experimental ecology was most interesting and made very enjoyable reading. He not only presented the current status of the field but he also provided valuable insight into the application of the scientific method to the study of natural communities. In his discussions on experimental manipulation of the physical and biological environment he stressed the value of accurately describing the ecosystem under study: "unless the original description is accurate and comprehensive the hypotheses and experiments will be of no use." In making a distinction between field and laboratory experiments as they apply to the understanding of natural ecosystems, he notes that results from field experiments can be more directly applied to natural ecosystems because biological interactions and the natural variation in the abiotic environment are included in the experiment.

In his chapter on in situ studies of animal behavior Herrnkind also emphasizes the importance of field studies and argues that aquaria are inadequate representations of the marine environment. He also notes that in situ studies frequently reward the investigator with observations of new or unknown behavioral phenomena. This well-organized chapter describes the many technological aspects of underwater research. He discusses the advantages and disadvantages of making direct observations of behavior using snorkel and mask, SCUBA, rebreathers, and habitats. In addition, he also indicates many of the potential dangers (air embolisms, nitrogen narcosis, oxygen toxicity, etc.) divers must consider when embarking upon a program of underwater study. In this respect I was somewhat surprised to note that no mention is made of osteonecrosis, a potential health problem with long-term effects. The rest of the chapter is devoted to methods of indirectly observing animal behavior including photography, television, passive acoustic monitors, telemetry, and sonar.

The more biologically orientated students frequently find the fields of marine biochemistry (Kittredge) and toxicology (Russell and Brodie) difficult to read with comprehension. However, the authors of these chapters have lucidly communicated their ideas and interests. Russell and Brodie have summarized current methods employed to study marine toxins by selecting a representative experimental design and leading the reader through it in a step-by-step study of a fish venom. The authors emphasize the importance of embarking upon such studies with an overall experimental design, for this stimulates the development of a logical progression of ideas about the unknown compound in question.

Kittredge's chapter on marine biochemistry also has a very personal flair. After delimiting marine biochemistry as "any problem which excites a marine biochemist" he discusses many interesting problems ranging from the formation of manganese modules to crab sex pheremones. He concludes the chapter with the fascinating observation that minute traces of petroleum inhibit the normal responses of crabs to the sex pheremones and speculates that the most detrimental effect of petroleum pollution could be an interference with the organisms' ability to detect food or sex partners.

Of the three remaining chapters two are concerned with physiology. Laverack's chapter on the neurophysiology of marine invertebrates appears comprehensive. While students of physiology could obtain valuable insight from reading it, the nonspecialist may find it a little detailed.

Fingerman attempts the formidable task of covering most aspects of the comparative endocrinology of marine invertebrates and fish. Insofar as the fish are concerned, the need for brevity has resulted in a sketchy presentation with the omission of a number of referen[illegible]

The final chapter of the book is almost entirely devoted to the use of the marine algae *Fucus* and *Acetabularia* as model systems for studying developmental biology (Quatrano). A chapter on plants in a book largely concerned with animals is encouraging and suggests that considerable thought went into the planning of this book as a whole.

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(R72)

The Salmon: Their Fight for Survival. *Anthony Netboy. Houghton Mifflin Company, Boston, Mass. 1974. 613 p., 33 figures and append. $15.00.*

Professor Netboy provides for the first time a global overview of the Atlantic and Pacific salmon and the steelhead trout. His perspective has the depth of time, reaching far back to the salmons' origins and to their earliest contact with man. His central theme is the impact of industrialized society on some of nature's most graceful and bountiful creatures. The drama unfolds as a tragedy which leaves the reader with a sense of impending further disaster.

He sets the stage with a review of our current interpretation of the prehistoric origins of the salmon and trout, and moves to their modern life histories and distribution in fresh waters and the world oceans. Then begins the tragic story of the European salmon from earliest historical time to the present: their early abundance and gradual disappearance from the Mediterranean countries and Portugal, and the near or total decimation of once plentiful stocks in Spain, France, England, Belgium, Holland, Germany, Denmark, Poland, and Finland. Only in Ireland, Scotland, Norway, Sweden, Iceland, and the USSR are the stocks still relatively abundant. Even here in most cases they are maintained only by constant vigilance and considerable industrial forbearance. Over all of Europe salmon stocks are either threatened or already extinct as a consequence of the Industrial Revolution and uncontrolled fishing in both the rivers and on the near and high seas.

In eastern North America, the European story is repeating itself. United States stocks have been brought almost to extinction by industrialization and indifference to what was thought by the early white settlers to be an inexhaustible bounty. In Canada the stocks are more numerous but again the trend, in historical perspective, is much the same — eastern Canada is simply at an earlier stage of industrial development.

The Pacific salmon and steelhead face three major threats in the southern parts of their range: diversion of rivers needed to bring the arid California soil to its potential fertility, damming for hydroelectric power to meet the energy needs of expanding industry, and the use of rivers to provide inexpensive sewers to the ocean. These demands on the fresh waters of California, Oregon, Washington, and Japan have decimated the natural stocks in the southern areas of their ranges in both the eastern and western Pacific. Stocks are maintained at their present levels only by massive commitments to artificial propagation.

So far, British Columbian, Alaskan, and Siberian stocks have been spared to a large degree the dispoiling of their freshwater environment by industrial development. Although the stocks still show the scars of past overexploitation in coastal fisheries, the most urgent concern is now overexploitation on the high seas. Professor Netboy points out that as the Russians reclaimed their coastal salmon fisheries from the Japanese who had occupied shore stations on Sakhalin, the Kurils, the Sea of Okhotsk, and East Kamchatka, the Japanese extended their fisheries onto the North Pacific and the Bering Sea. The effects are felt most by Siberian chum and pink salmon stocks which occupy these ocean areas, but the once-abundant Bristol Bay sockeye of Alaska are also jeopardized. The high-seas harvest consists partly of immature fish representing a waste of potential yield, and fish of mixed origin, making it impossible to regulate the fishery according to each stock's reproductive needs.

Professor Netboy's presentation is exceedingly easy to read, so much so that one might suspect him of overlooking technical accuracy to make his text more readable. With few exceptions such is not the case. The work is well documented and interested readers will find the extensive bibliography a most useful way to explore the more detailed literature. The book also contains an appendix of fishery statistics presented in such a way as to make it possible to assess recent trends in salmon abundance at a glance. There is a short section on present-day availability of sport fishing for salmon and steelhead in Europe and North America, and one for the gourmet on salmon cookery past and present.

Professor Netboy's work achieves the nearly unattainable — it will satisfy both the layman and the expert. Now neither can say that he is fully knowledgeable about the world's salmon unless he has read this fine book.

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The Inland Waters of Tropical Africa. An Introduction to Tropical Limnology. *L. C. Beadle. Longman, London, Don Mills, Ontario, 1974. viii + 365 p. £7.00, $19.00 Canadian.*

Of all tropical land masses Africa is perhaps the most interesting to the limnologist. While its great rivers are rivalled but not diminished in appeal by those elsewhere, its diversity of lakes is unique. These include the whole range from continuously mixed to permanently stratified, and some are now well known for their rich endemic faunas. Add to these some of the world's largest tropical swamps, a host of small water bodies of diverse character, and some of the greatest of the world's man-made lakes and the scope of Professor Beadle's book, which seeks to introduce students to both the physical and biological aspects of tropical limnology, can be appreciated. On this broad canvas he has managed, like some of the Dutch painters, to provide a wealth of detail while maintaining an overall unity of purpose.

While a prime aim of the book is to serve as an introductory text to students in Africa, its appeal

should be far wider, for there can be few limnologists who will not learn something new by perusing its pages, and indeed those lacking previous background information may find some of the early chapters rather hard going. These are devoted to historical aspects and basic principles as applied to tropical regions where conditions differ in many ways from those in the more familiar temperate waters. Here the reader is introduced to some of the basic concepts of physical limnology and of productivity, often illustrated by African examples, and makes the acquaintance of the rich African freshwater fauna. Beadle is not one who unthinkingly accepts the conclusions of others, and takes an early opportunity to advise the reader to adopt a critical attitude to all numerical data and later does an excellent job in debunking the belief that simple correlations can be found between the chemistry of a water body and its fauna and flora. He rightly points out that the apparently insignificant ecological effects of ionic differences on freshwater animals should not surprise us as freshwaters are chemically variable and unstable and could only have been colonized by organisms with ionic regulating mechanisms that can function under a wide range of chemical conditions. Africa provides examples of waters over an enormous range of salinities and ionic composition, yet within the salinity range 0.02–2.5‰ only the level of calcium seems to be limiting in very dilute waters. Those familiar with other tropical regions might feel that the other side of the coin could perhaps have received more attention here. For example, the faunas of the "black" and "white" waters of the Amazon sometimes differ strikingly, but again low calcium levels and low pH values in the "black" waters may be the answer.

The reader is then taken on a limnological tour of the continent, visiting the great rivers and lakes and many other places of limnological interest to which Beadle proves to be an admirable guide. At every step one learns something of interest, such as why, in spite of being a closed basin, Lake Chad remains fresh; about the store of methane in the depths of Lake Kivu; or how the Upper Lualaba River might have acquired Nilotic fishes. The tour includes a visit to the Sahara — a region that might be expected to offer little to the limnologist — but which not only has several water bodies of considerable intrinsic interest and large reserves of subsurface water but was much wetter in the recent past. The hydrological changes that have taken place there since the mid-Pleistocene are the key to our understanding of certain curious biological features in the area to the south of the present desert.

Past geological and climatic changes and their hydrological consequences indeed receive considerable attention, and with good reason, for only by understanding these can we appreciate the significance of some of the biological features of the present-day lakes and rivers. It must be remembered that while much of the limnological work carried out in the north temperate zone has been in areas that only about 10,000 years ago were covered by ice or subject to arctic conditions, many African water bodies have a much longer history, some of the consequences of which are readily apparent. Indeed one feels at times that the author is more interested in the history of the various basins considered than in the organisms to be found there. For example, while the account of Lake Victoria is interesting and informative it tells us rather little about the ecology of the many animals living there. Lake Tanganyika fares better and it is particularly gratifying to see the morphological features of its 'thalassoid' molluscs explained in terms of adaptation to physical conditions rather than by unsupported appeal to peculiarities of water chemistry.

Ecology is not, however, neglected and the account of swamp life for instance is, as one might expect from Beadle's interests, full of exciting information. Some of this is of considerable practical value, such as the way in which swamp clearance can actually lead to an increase in malaria-carrying mosquitoes, and of the importance of aestivation in snails (with which schistosomes can also survive) in the epidemiology of bilharzia. The practical value of African fisheries is also mentioned, though not in detail, and what appears to be a somewhat over-optimistic view is taken. One regrets the failure to condemn the introduction of the predatory *Lates* into Lake Victoria where the main objection was not (as claimed) that it might eat *Tilapia*, but that it is ecologically inefficient to introduce predators whose prey can be harvested directly. One is also puzzled by the statement that there are no signs as yet that the *Tilapia* fishery has thereby suffered, when in fact, by other means, *Tilapia* stocks have been wantonly depleted. Attention is also drawn to the opportunities provided to the limnologist by man-made lakes and to the problems they pose. The latter are sometimes of great practical importance.

The author modestly says this is not a comprehensive treatise, but the bibliography should lead the enquiring reader to more detailed studies of particular regions or phenomena. While no one will agree with all that is said, as a background to African limnology this book has no rivals and, very important in a work of this sort, it succeeded in holding at least this reader's interest throughout. It is, therefore, a pity that one has to give a warning about the rather large number of small but irritating errors with which it is marred. For example, the names of several organisms are wrongly spelled, some being spelled differently in different places; *Neritina* is a gastropod not a bivalve mollusc; *Lironeca tanganyikae* is a parasite of a cichlid, not a clupeid, fish (a matter of some importance in the thesis developed); it is branchiurans of the genus *Argulus*, not copepods of the genus *Lernaea*, that have produced seven endemic species in Lake Tanganyika; the mystery of the supposed endemic cichlid of West African affinities in Lake Rudolf (*Pelmatochromis exul*), now known to be a misidentified *Hemichromis bimaculatus*, had been cleared up in time to prevent the perpetuation of an error of long standing; the Kafu River is on one page given as the Kafue — a very different stream; and one looks in vain in figure 7.4 for some of the information said to be portrayed there. It is hoped that when

a second edition is called for, such errors will be rectified, and one wishes the volume the success it deserves.

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The Benthos of Lakes. *Ralph O. Brinkhurst. The Macmillan Press, Ltd., London and Toronto. St. Martin's Press, Inc., New York, N.Y., 1974. xii + 190 p., $45.95.*

The dedication, "To all those who have hauled an Ekman by hand from 50 m in a small boat on a windy day to find a stone caught between the jaws" — an expression of ultimate frustration painfully familiar to benthologists — aptly introduces the subject of this treatise.

The volume is primarily a review of the substance, complexities, and inadequacies of lacustrine benthic ecology. The work is well organized, copiously illustrated, and replete with citations as attested by the 17-page bibliography. Editorial oversights (e.g. no text reference to figures 2.2 and 2.3) are uncommon. A theme implicit throughout is that the book can serve as a guide for modeling lake ecosystems, although the author cautions that the construction of models must not be an end in itself, only a means by which we can achieve predictability in lake dynamics.

Following the preface and a short glossary, the reader finds the book organized into three principal parts "The Distribution and Abundance of Lake Benthos," "Sources of Variability," and "Discussion and Conclusions." Appropriately, Part I begins with a brief historical account of trends in lentic benthology. This is followed by a discussion of the attempts to classify lakes according to their macroinvertebrate components (too often on the basis of the Chironomidae, which the author recognizes as a poor criterion) or by the bathymetric alignment of species and their abundance. A discourse on biomass production is contributed by M. G. Johnson and a chapter on community structure and energy flow in the benthic ecosystem is authored by A. V. Tyler, who draws on examples from marine, terrestrial, and limnetic environments.

In Part II of the book, "Spatial and Temporal Complexity" receives most of the author's attention. In this chapter he endeavors to describe many of the complexities involved in the holistic approach to understanding the benthic ecology of lakes. Contributing to these complexities is spatial and temporal variation. One perplexing source of variability in quantitative and qualitative estimation of benthic biocoenoses is attributed to the inherent inefficiency of some kinds of sampling devices. Sampling variability that results from the method employed in sieving a bottom sample — sometimes the greatest source of error — is, unfortunately, mentioned only briefly. It is very difficult, if not impossible, to standardize the manner in which benthologists reduce a sample to a desirable residue. The amount of washing to which a sample is subjected assuredly influences the number and kind of animals lost in the process, as does the composition of the sediments that were sampled. The problem deserves much more attention than the author gave it.

In his conclusions (Part III) regarding the direction benthological research should take, Brinkhurst recognizes the dichotomy between the more recently popular holistic approach (the laudable studies on Lake Borrevann in Norway and Võrtsjärv in Estonia, for example) and the historically popular reductionist approach. He professes no strong preference for one or the other but remarks that it would be unwise to emphasize the former at the expense of the latter.

Into the midst of seemingly infinite involvement of mankind in a finite aquatic environment, *The Benthos of Lakes* is a timely source book. For the fishery biologist, pollution ecologist, public health engineer, and student, among others, it is a valuable guide toward uniformity of approach in benthological studies. Unfortunately the high price may prevent the book from becoming an addition to personal libraries.

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The Blackbass in America and Overseas. *William H. Robbins and Hugh R. MacCrimmon. Biomanagement and Research Enterprises, S. S. Marie, Ontario. vi + 196 p. $7.95 paperback, $12.95 hard cover.*

The authors of this book bravely suggest that it should "become a valuable reference on the bookshelves of all those persons privileged to study, manage or fish for the fabulous blackbass in America or overseas." The compromises necessary to provide something for everybody in such ambitious undertakings usually result in providing something less than satisfactory for everybody, and this book proved no exception.

The strength of the book lies in its detailed distribution data. This information will be useful for further studies, but it is not well synthesized and makes little contribution to the zoogeography of the basses. From the point of view of the manager there is equally scant basis for predicting where to transplant or to not transplant bass to be found in the dismal chronicle of man's past efforts in this area.

The chapter that deals with taxonomy is of little scientific interest because it does not cover the full reach of the systematic problems. Nomenclatural histories, moreover, are of little interest to the angler or fishery manager. Those groups may be confused, on the other hand, by the unexplained return to an

archaic set of common names (blackbasses) for the six species. A glossary of scientific terms used in this and other sections would have been helpful to the lay reader.

The sections and chapters on biology and habitat requirements contain a great deal of information that will appeal to the interested sportsman. The manager or scientist, however, would have benefitted more from comparative summaries of available data on such things as growth, fecundity, incubation rates, geographic differences in spawning times, etc. for each species. There could also have been more attention given to biocoenoses.

The whole area of population dynamics is largely ignored, and this is where the interest of the fishery managers chiefly lies. Oscillations in stock abundance, for example, are of great concern, and the ample literature on year-class strength variation in largemouth and smallmouth bass was given short shrift. Regulation of the bass harvest was particularly poorly treated. There is a good literature on production, cropping rates, and mortality schedules that could have been usefully summarized for the fishery managers. There are references to overfishing in the book, but the essential problem for the managers has been to determine allowable yield, in the face of obviously extreme environmental control.

The book omits "how to" details on angling, and at the same time offers only generalized information concerning "where and when." This was probably deliberate since Byron Dalrymple's book (Modern Book of the Black Bass) was adequate to these purposes.

In its overall impact, the book was curiously anachronistic, and one wonders if its net effect may not be a negative one. It refers, for example, to a "relatively recent trend towards liberalized bass management." This concept is of course far from recent, and in fact, thinking has been moving in the opposite direction for several years. The thinking with regard to transplantation has similarly changed.

Introducing exotic fish species for many years epitomized managerial failure, and some extraordinary mistakes with long-lasting effects both on native faunae and the practice of fishery biology, were perpetrated. Nowadays the management approach is trending towards attacking causes rather than symptoms. In fairness, one section of the book advises caution and adequate biological information before attempting introductions of the bass species. Its silence overall on the questions of the ethics and wisdom of messing with ecosystems we don't understand, however, must be interpreted as consent.

The book is well organized and presented. The editing has been efficient and the quality of the printing is good. Aside from a tendency towards adjectival overkill, it is quite readable. The graphics are of generally high quality except for the range maps, where area shadings are very poorly differentiated.

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(R46)

Light's Manual: Intertidal Invertebrates of the Central California Coast. Third edition. *Ralph I. Smith and James T. Carlton [ed.] University of California Press, Berkeley, Los Angeles, and London, 1975. xviii + 716 p. $20.00.*

When Professor S. F. Light died in 1947, his *Laboratory and Field Text in Invertebrate Zoology* was less than six years old. This unpretentious work consisted of keys for the identification of invertebrates of the coast of central California and some suggestions for field studies that could be carried out in that region. In 1954, the manual was successfully revised by a number of collaborators who attempted to perpetuate the spirit of Dr Light's approach. Since then, so much has been done with systematics, ecology, and other aspects of the biology of invertebrates on the Pacific coast that a further revision has been impatiently awaited. For this huge undertaking, Ralph Smith, who played an important role in getting out the second edition, was joined by James Carlton and more than forty specialists concerned with particular groups.

The new *Light's Manual* is really new. All sections have been completely updated. There is an opening chapter that deals with generalities of intertidal life and suggestions for field study. This is followed by an interesting account of introduced species that have become established in central California. The rest of the book covers the various phyla, classes, and orders of intertidal invertebrates, plus intertidal fishes, eelgrasses, and common seaweeds. In general, each section consists of an illustrated explanation of terminology, a key, an annotated checklist, and a bibliography. Some groups, especially those that are poorly known or that consist of numerous small species not likely to be dealt with except by a specialist, are not accorded a key. At least a brief statement about the group is provided, however, to indicate the state of our knowledge about it. In addition to the drawings that illustrate terminology, there are many other figures that show the key characters of genera and species.

A valuable feature of the book is its coverage of the literature on invertebrates of the Pacific coast. The bibliography at the end of each section includes pertinent taxonomic monographs and papers. It also lists treatises and advanced textbooks with which anyone contemplating work on a particular group should be familiar. The annotated checklists, which provide helpful information on habitats of intertidal invertebrates represented in central California, include further references, mostly concerning individual species. It is here that one finds leads to hundreds of studies on behavior, reproductive biology, symbiotic relationships, and so on.

This book was worth waiting for. We should continue to hope that the invertebrate fauna of other regions of North America will eventually be covered as thoroughly. But just remember that if it had not been for the devotion and industry of Dr Light and the students who helped him with the first edition, we

would not be this far along with what we know about the marine invertebrates of central California.

The University of California Press merits recognition for putting out *Light's Manual* in an attractive and functional format, and at a sensible price.

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Culture of Bivalve Molluscs: 50 years experience at Conwy. *P. R. Walne. Fishing News (Books) Ltd., West Byfleet, Surrey, England, 173 p. £5.85.*

This slim and expensive paperback will disappoint the reader expecting a discussion of the sociology of bivalve moluscs, but as a lucid and thorough introduction to the rearing of the European flat oyster, with notes on other species, it is highly recommended. It should be noted, lest the editorial competence of this journal be questioned, that Conwy is the accepted spelling for Conway, another example of the current preoccupation with ethnic minority sensitivities.

The European experience of bivalve rearing in enclosed environments originated at Conway in 1917 when oyster spat were found in tanks used for mussel purification. The discovery initiated organized attempts to produce a stable supply of young stock to service the British oyster industry, which suffered from irregular recruitment and the effects of pollution.

The book is based on a series of popular lectures delivered under the auspices of the Buckland Foundation, which may account for the pleasing style and lack of scientific obscurantism. Initial chapters deal with the gross anatomy and nutritional physiology of oysters and larval growth in natural and enriched sea water. The bulk of the book is concerned with hatchery rearing, that is, the growth of larvae in filtered or sterilized sea water and their growth utilizing chemostat cultures of microorganisms. It is clear that, despite years of experience, bacterial contamination of food organisms cultures and the larvae remains a problem, "solved" at Conway by the massive use of antibiotics.

While this work is essentially parochial, reporting only work undertaken at Conway, and chiefly concerned with the flat oyster (*Ostrea edulis* L.), it is a useful contribution summarizing techniques and provides a guide to the important scientific literature in short end-of-chapter bibliographies. It is recommended with confidence to those interested in the practical aspects of bivalve mollusc husbandry. The extortive price is offset by valuable advice on how to avoid those pitfalls learned by practical, as opposed to experimental, experience.

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(R68)

Optimum Sustainable Yield as a Concept in Fisheries Management. (Proceedings of a symposium held during the 104th Annual Meeting of the American Fisheries Society, Honolulu, Hawaii, September 9, 1974.) *Philip M. Roedel [ed.] Special Publication No. 9, American Fisheries Society, Washington, D.C., 1975. vii + 89 p. $3.00.*

This symposium was held at the 104th Annual Meeting of the American Fisheries Society in Honolulu, Hawaii, on September 9, 1974. Although it dealt rather superficially with the latest villains (maximum sustainable yield and maximum economic yield) and heroes (optimum sustainable yield and optimum yield), a number of telling blows were struck for the open acknowledgment and incorporation of economic, social, and political factors in the management of fisheries resources — marine and freshwater, recreational and commercial. The Summary and Critique contains excellent definitions of Optimum Yield and Optimum Sustainable Yield.

A broad spectrum of individuals participated, and their evident earnestness was refreshing. Hopefully, the message will disseminate, particularly to legislative bodies, who must act before any real progress can be achieved to improve the management philosophy of the various regulatory agencies. Equally refreshing would be a similar symposium that would constructively reveal the weak foundation of basic knowledge in the fields of biology, economics, political science, and sociology, and its implication with respect to realistic precision in quantitative management.

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Books Received / Livres reçus

July–August 1975

*__Ecology, Utilization, and Management of Marine Fisheries.__ *George A. Rounsefell. The C. V. Mosby Company, St. Louis, Toronto, Ont., 1975. xi + 516 p. $26.25.*

(Textbook stressing importance of the environment and its effect on abundance, distribution, and life history adaptations of selected commercial species.)

JIBP Synthesis. Volume 10. Productivity of communities in Japanese Inland Waters. *Edited by S. Mori and G. Yamamoto. Japanese Committee for the International Biological Program, University of Tokyo Press, Tokyo, Japan, 1975. vii + 436 p.*

(Estimation of productivity in eight inland-water areas in Japan and one in Malaysia.)

*__Marine Geotechnology. An International Journal of Seafloor Science and Engineering.__ *Edited by Adrian F. Richards. Crane, Russak & Company, Inc., New York, N.Y., 1975. Vol. 1, No. 1, viii + 71 p. Subscription price $34, $17 for individuals.*

(New quarterly journal covering theory and practice of geotechnical ocean engineering and geotechnical aspects of marine geosciences.)

*__Marine Science Communications. Volume 1, No. 1.__ *Edited by D. W. Hood. Marcel Dekker, Inc., New York, N.Y., 1975. ii + 100 p. Subscription price $35.00.*

(Bimonthly journal emphasizing publication of articles that are timely and relevant in basic, applied, and ocean systems.)

*__Models in Ecology.__ *J. Maynard Smith. Cambridge University Press, Cambridge, London, New York, Melbourne, 1974. xii + 146 p. $12.25*

(Presents mathematical and laboratory models aimed at an understanding of the general properties of ecosystems, including predator–prey systems, competition, migration, stability and complexity, coevolution, and territorial behavior.)

Oceanographic Data Reduction Manual I. *Pierre G. Dallemagne. MSS Information Corporation, New York, N.Y., 1975. 296 p. $5.00.*

(Provides instructions for reducing physical, meteorological, and chemical data and encoding them in accordance with the National Oceanographic Data Center's storage retrieval system.)

*__Principles of Fishery Science.__ *W. H. Everhart, A. W. Eipper, and W. D. Youngs. Cornell University Press, Ithaca, New York, 1975. 288 p. $12.50.*

(Comprehensive survey of methods and concepts basic to managing fish populations; textbook aimed primarily at graduate and upper division undergraduate students.)

Respiration of Marine Organisms. Proceedings of the Marine Section, First Maine Biomedical Science Symposium, March 14–15, 1975, Augusta, Maine. *Edited by J. J. Cech Jr., D. W. Bridges, and D. B. Horton. The Research Institute of the Gulf of Maine (TRIGOM), South Portland, Maine, 1975. 211 p. $4.00 paperback.*

(Seventeen contributions on general physiological/pharmacological, cell biological/biochemical and clinical aspects of respiration of phytoplankton, invertebrates, and fish.)

*To be reviewed.

Paul-E. Gagnon

1901 - 1975

Paul-E. Gagnon, D.Sc. FCIC, membre de l'Office des recherches sur les pêcheries du Canada de 1950 à 1960 est décédé récemment à Montréal. Né à Kingsey, Québec en 1901, il obtenait un B.Sc. de l'Université Laval en 1926 et un D.Sc. de la Sorbonne en 1929. De retour au Canada, il commençait une longue carrière dans l'enseignement et la recherche à l'Université Laval. Nommé professeur au département de Chimie en 1931, il en devient le directeur en 1938, poste qu'il occupe jusqu'en 1951. Il prend charge également, de 1940 à 1945, du nouveau département de Génie chimique, en même temps qu'il se voit confier la direction de l'École des Gradués. Après 20 ans à ce dernier poste, il quitte l'Université Laval en 1960 pour se rendre à Vienne comme directeur de la division des échanges de la Commission internationale de contrôle de l'énergie atomique. Rentré au pays quelques années plus tard, il est nommé adjoint spécial au rédacteur en chef des journaux scientifiques au Conseil national de recherches du Canada. Durant sa fructueuse carrière, il est l'auteur de plus de cent publications scientifiques, il exerça de nombreuses fonctions au sein de sa profession, entre autres: secrétaire de la Société de Chimie du Québec, président de la Société de Chimie du Canada, membre du Conseil national de recherches, membre de la Commission de contrôle de l'énergie atomique, membre du Conseil de recherche pour la défense, président de l'Institut de Chimie et président de la Société royale (section III). Plusieurs groupements scientifiques canadiens et étrangers de même que des universités ont tenu à reconnaître ses mérites et les services rendus à sa profession. Il était ainsi professeur émérite à la Faculté des Sciences et de Génie de l'Université Laval, docteur honoris-causa des universités McMaster et Toronto, membre à vie de la Chemical Society of London, honorary-fellow de l'Institut de Chimie du Canada et membre-à-vie de l'Institut des Ingénieurs canadiens.

GABRIEL FILTEAU

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COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Movement of yellow perch (*Perca flavescens*) and white sucker (*Catostomus commersoni*) in a nearshore Great Lakes habitat subject to a thermal discharge **J. R. M. Kelso**

Production by brook trout (*Salvelinus fontinalis*) in four streams in the Matamek watershed, Quebec **J. F. O'Connor and G. Power**

Changes in ouabin-sensitive adenosine triphosphatase activity in gills of coho salmon (*Oncorhynchus kisutch*) during parr–smolt transformation **M. A. Giles and W. E. Vanstone**

Field determination of the critical nutrient concentrations for *Cladophora* in streams **S. L. Wong and B. Clark**

Ration, growth, measures of somatic and organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis **A. V. Tyler and R. S. Dunn**

Physiological changes during the course of blooms of *Aphanizomenon flos-aquae* **F. P. Healey and L. L. Hendzel**

Isolation and identification of blue-green algae producing muddy odor metabolites, geosmin, and 2-methylisoborneol, in saline lakes in Manitoba **J. L. Tabachek and M. Yurkowski**

Optimal exploitation of multiple stocks by a common fishery: a new methodology **R. Hilborn**

Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock **C. B. Schreck, R. A. Whaley, M. L. Bass, O. E. Maughan, and M. Solazzi**

Some factors affecting rainbow trout (*Salmo gairdneri*) predation on migrant fry of sockeye salmon (*Oncorhynchus nerka*) **R. M. Ginetz and P. A. Larkin**

Purging a muddy–earthy flavor taint from rainbow trout (*Salmo gairdneri*) by transferring to artificial and natural holding environments **D. G. Iredale and R. K. York**

Adaptive control of fishing systems **C. J. Walters and R. Hilborn**

Chronic effects of reduced pH on brook trout (*Salvelinus fontinalis*) **R. Menendez**

The eosinophilic cell of the white sucker, *Catostomus commersoni* **R. J. G. Lester and B. A. Daniels**

Terrigenous organic matter in surface sediments from the Gulf of St. Lawrence **R. Pocklington**

Surface circulation pattern in the Gulf of St. Lawrence **M. I. El-Sabh**

Mean value and variability of fish catches in fluctuating environments **M. Gatto and S. Rinaldi**

Coastal circulation and physical oceanography of the Scotian shelf and the Gulf of Maine **W. H. Sutcliffe Jr., R. H. Loucks, and K. F. Drinkwater**

Upwelling along the Yarmouth shore of Nova Scotia **C. J. R. Garrett and R. H. Loucks**

Quantifying nutrient requirements of fish **I. H. Zeitoun, D. E. Ullrey, W. T. Magee, J. L. Gill, and W. G. Bergen**

Notes

Blood lactate levels in free-swimming rainbow trout (*Salmo gairdneri*) before and after strenuous exercise resulting in fatigue **W. R. Driedzic and J. W. Kiceniuk**

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Journal of the Fisheries Research Board of Canada

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We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

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Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

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Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

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Journal de l'office des recherches sur les pêcheries du Canada

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Lettres: Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

Dispositions générales

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de reproductions photographiques ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauter lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves; il doit être retourné en même temps que celles-ci, non pas au bureau de la rédaction, mais au Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8, qui se chargera de la facturer. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

Listes de contrôles pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphs son-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal of the Fisheries Research Board of Canada

Guide for Typists Preparing Copy

Read "Guide for Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C). Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send a photographic reproduction (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal de l'office des recherches sur les pêcheries du Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21,5 × 28 cm (8,5 × 11 pouces), laissant toujours une marge d'au moins 2,5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les miniscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, ne nom et l'affiliation des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit un reproduction photographique (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir Appendice E). Les noms de revues sont abrégés suivants les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Dissemination of Scientific and Technical Information

New and improved ways of recording and communicating scientific and technical information will soon be described in a special guidebook supported by the National Scientific Foundation of the United States. The Guide's purpose will be to provide practical information about how to disseminate scientific and technical information more effectively and efficiently. The Guide will describe innovations and improvements that have been selected for their practicality, potential for improving communication, and ability to reduce costs.

The Guide will cover the full range of conventional publishing activities. In addition, it will cover print-on-paper, mixed-media, and nonprint alternatives to conventional methods of dissemination; these alternatives will include microfilms, magnetic tape, audiovisual media, and on-line computer networks.

The National Science Foundation has awarded a contract to Capital Systems Group, Inc., of Rockville, Maryland, to produce and distribute this Guide, which is intended for use by publishers, editors, and others responsible for communication programs. It will be entitled *Improving the Dissemination of Scientific and Technical Information: A Practitioner's Guide to Innovation*. The Guide is the product of a one-year study, in which Capital Systems Group contacted hundreds of people and organizations around the world, compiling information concerning developments that might be used to improve the dissemination of information.

Persons who are interested in obtaining subscriptions to the Guide, or who have information concerning improvements that might be included in it, are urged to contact John M. Strawhorn, Capital Systems Group, 6110 Executive Boulevard, Suite 850, Rockville, Maryland 20852 (telephone 301–881–5266).

Communique de Presse

Des méthodes nouvelles et améliorées de compilation et de dissémination de l'information scientifique et techniques seront décrites sous peu dans un manuel spécial financé par la National Science Foundation des Etats-Unis. Le Guide donne des directives pratiques sur la façon de disséminer plus efficacement l'information scientifique et technique. Il contiendra des innovations et des améliorations choisies à cause de leur aspect pratique, de leur potentiel pour de meilleures communications et des moyens qu'elles offrent de réduire les coûts.

Le Guide couvrira l'éventail complet de la publication conventionnelle. Il traitera de plus de l'imprimé sur papier, des média mixtes et des alternatives, autres que l'imprimé, aux méthodes conventionnelles de dissémination; ces alternatives comprennent microformes, bandes magnétiques, média audio-visuels et ordinateurs sur réseaux.

La National Science Foundation a accordé un contrat au Capital Systems Group, Inc., de Rockville, Maryland, pour la production et la distribution de ce Guide, qui s'adressera aux éditeurs, directeurs de publications et autres personnes responsables de programmes de communication. Il aura pour titre *Improving the Dissemination of Scientific and Technical Information: A Practitioner's Guide to Innovation*. Le Guide est le résultat d'une étude d'un an, au cours de laquelle le Capital Systems Group est entré en rapport avec des centaines de personnes et organisations dans le monde entier, compilant des renseignements sur tout développement susceptible d'améliorer la dissémination de l'information.

Les personnes intéressées à se procurer le Guide ou qui auraient des suggestions concernant certaines améliorations qui pourraient y être incluses sont priées de communiquer avec John M. Strawhorn, Capital Systems Group, 6110 Executive Boulevard, Suite 850, Rockville, Maryland 20852 (téléphone 301-881-5266).

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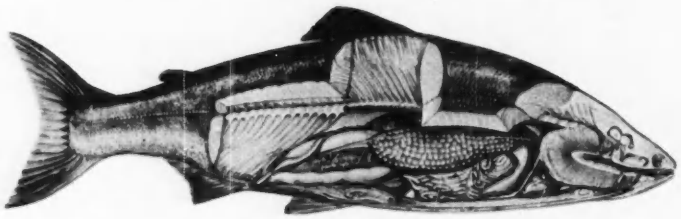

A Practical Guide To The Anatomy And Physiology Of Pacific Salmon

L.S.Smith
G.R.Bell

Fisheries workers and the general public often need or wish to locate and identify special parts of the salmon to describe abnormalities or to submit appropriate samples rather than the whole fish for examination by specialists, but there is no single document describing the gross anatomy or general functioning of Pacific salmon species. In this publication the gross anatomy of Pacific salmon is discussed and illustrated with drawings and photographs. Aspects of anatomy and physiology of special interest to fisheries biologists are emphasized, and the cardiovascular system has been examined in some detail using angiograms and rigid plastic casts of the blood system. There is only scant discussion of the anatomy of the skeleton, muscles, and nervous system. Most of the information could also apply to Atlantic salmon and to the trouts.

(Canada: $2.00, Other countries: $2.40; Cat. No. Fs 4-31/27; 14 pages)

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